Biotic interactions and edaphic variation modulate geographic range limits in *Clarkia xantiana* ssp. *xantiana*

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Introduction

The study of species' geographic distributions, especially limits to those distributions, lies at a fruitful nexus of ecology and evolutionary biology. At these distributional limits, the ecological interactions that determine population mean fitness across and beyond the range limit collide with the evolutionary limits to adaptation. Species' geographic distributions comprise the spatial extent of their populations, and vary greatly in size, shape, and the arrangement and abundance of populations contained therein. However variable these distributions are, they all are bounded by an invisible perimetric line on the landscape beyond which populations of that species cannot be found, i.e., the species' geographic range limit. Why are individuals able to persist on one side of this border but are excluded from regions directly adjacent? This deceptively simple question is a perennial one that underlies many foundational questions about ecological interactions and adaptation.

Distributions are structured by myriad factors with large, small, and interactive effects, but the essential determinants of the spatial patterns are relatively simple — populations persist where long term growth rates are equal to or greater than replacement ($\lambda \geq 1$). But given sufficient time and adequate heritable variation for ecologically important traits, species' ranges should theoretically be able to continually expand outward through sequential adaptation by populations at the range edge (see Kawecki 2008 for review). Of course, this is not the observed pattern in nature; most species are restricted to a relatively small fraction of the planet's available habitat.

Hypotheses regarding the formation of range boundaries invoke two broad causal factors: a failure to disperse outside the range boundary or a failure to adapt to novel conditions existing therein (Geber 2008; Sexton et al. 2009; Geber 2011). The former occurs when a species' distribution is in disequilibrium with its environmental niche – i.e., it is not occupying all of the suitable habitat available to it. This may occur if there are physical barriers to dispersal, such as oceans or mountain ranges, or if the rate of dispersal does not keep pace with the rate at which suitable habitat becomes available (e.g., recolonizing after glacial retreat: Svenning et al. 2008). Barring dispersal lags,

range limits will reflect maladaptation to environments outside the distributional boundary. The latest theoretical treatments of evolutionary constraints on species' ranges highlight how the demographic costs of maladaptive gene flow from central populations can cause genetic drift to overpower selection and lead to abrupt range limits (Polechová 2018). Steep, or particularly, non-linear environmental gradients seem to be especially important in limiting adaptation at range limits (Polechová 2018; Bridle et al. 2019).

Questions concerning the determinants of species' distributions have a long history, but the field has seen a recent surge of interest as researchers try to predict species' responses to contemporary environmental perturbations such as climate change. Most species' range boundaries do not align with any obvious physical barrier to dispersal, indicating that evolutionary constraint or dispersal lag are likely the more widespread phenomena structuring these range boundaries. Evidence for adaptive limitation is corroborated by transplant studies showing decreases in fitness beyond a species' range boundary (e.g., Eckhart et al. 2004; Angert & Schemske 2005; Geber & Eckhart 2005; Griffith & Watson 2006), and meta-analyses support the notion that maladaptation is limiting colonization outside many species' current range boundaries (Hargreaves et al. 2014; Lee-Yaw et al. 2016). If adaptation to novel environments is constraining range expansion, then the question becomes, *which* environmental variables are driving these patterns?

Most of the classic papers in range limit ecology focus on identifying associations between distributional boundaries and climatic variables (see Gaston 2003, Sexton et al. 2009 for review). With the advent of species distribution modeling, these analyses have increased greatly in number and sophistication, and spatially-explicit predictive models have been built for many species. The majority of these models correlate species occurrences to variables related to temperature and precipitation, often finding strong associations. But the usefulness of these models depends on the biological import of the factors included, and spatially autocorrelated climatic variables will rarely fail to correlate with similarly autocorrelated patterns of population occurrence across the landscape (see Fourcade et al. 2018). Indeed, the large variability in species' responses to recent warming (Lenoir et al. 2008; Chen et al. 2011; Rumpf et al. 2018) suggests that we

are likely missing other factors important in structuring distributions. However, the role of non-climatic factors in shaping the realized distributions of species remains sorely understudied.

Every species on earth interacts with a multitude of other species, and these biotic interactions have large effects on the ecology and evolution of participating organisms. Though the effects of biotic interactions on regulating species' geographic distributions are often assumed to be minimal compared to the effects of climate, there is accumulating evidence for plant species' distributional limits being influenced by competitors (e.g., Bullock et al. 2000; Ettinger and HilleRisLambers 2017), consumers (e.g., Bruelheide & Scheidel 1999; Baer and Maron 2018), and mutualists (e.g., Moeller et al. 2012; Afkhami et al. 2014). However, most of this evidence is, as with climatic variables, correlative, and there have been increased calls for more experimental investigations of the influence of biotic interactions on range limits (Van der Putten et al. 2010; HilleRisLambers et al. 2013; O'Brien et al. 2017). In addition, inclusion of edaphic (soil) factors can often greatly improve plant species' distribution models (Bertrand et al. 2012; Walthert and Meier 2017), and despite the obvious importance of soil environments on individual plant phenotype and fitness, their role in modulating plant species' range limits is largely untested (Thuiller 2013).

Most species' are likely to be distributed over complex environmental gradients comprising changes in multiple abiotic and biotic variables. Transplant experiments, ideally combined with manipulations of putatively important environmental variables, are the most powerful method to test the relative influence of these variables on the location of the species' range limit. In my dissertation, I use a variety of greenhouse and field experiments with the California native plant *Clarkia xantiana* ssp. *xantiana* (Onagraceae) to dissect multiple aspects of the hypervariable environment, quantifying their influence on plant lifetime fitness inside and outside the subspecies' geographic range limit (Fig. I.1).

C. x. xantiana is a subspecies well-suited to addressing these questions. Despite having no obvious barriers to dispersal, historical data indicate that *C. x. xantiana*'s range limit has not moved significantly for at least one hundred years (Eckhart & Geber 1999),

with population genetic analyses suggesting that populations at the range edge have remained relatively stable for at least several hundred generations (Moeller et al. 2011); thus, the system offers an excellent opportunity to examine factors that constrain range expansion in a subspecies whose distribution is very well characterized. Especially puzzling is *C. x. xantiana'*s failure to establish beyond its eastern range limit, where its sister taxon, *C. x. parviflora*, is able to persist. A large reciprocal transplant study showed that each subspecies' respective fitness is reduced when planted into the other's range, with the authors identifying several abiotic and biotic factors likely contributing to the maintenance of this border (Geber & Eckhart 2005). Water relations are thought to play a role in preventing eastward expansion of *C. x. xantiana* (Eckhart et al. 2011), but there is also evidence for increased herbivory (Geber & Eckhart 2005) and pollen limitation (Moeller 2006; Moeller et al. 2012) outside the eastern range boundary, indicating that abiotic and biotic factors can form complex environmental gradients that may synergistically affect range limit formation for *C. x. xantiana*.

Competition is by far the most studied interspecific interaction in range limit research (i.e., competitive exclusion) but represents only a fraction of the potentially important biotic processes occurring throughout the lifespan of a species. For plants, herbivory is another ubiquitous antagonistic interaction with widely documented effects on individual and population performance (see Maron & Crone 2006 for review), but there are few tests of its potential to constrain the geographic distributions of plant species. In *C. x. xantiana*, strong herbivory by small mammals on plants transplanted outside of the range (Geber & Eckhart 2005) suggests this may be another important factor in constraining expansion into the east.

In contrast to antagonistic interactions, positive interactions such as facilitation and mutualism are known to mitigate abiotic stressors in many organisms. Consequently, they have the potential to also affect population viability and the formation of range limits, but investigation of these factors in regard to geographic distributions is limited. If composition or abundance of mutualist communities varies within and outside a species' range, this variation in the biotic environment may play important roles in range limit formation. For the primarily outcrossing *C. x. xantiana*, there is evidence that *Clarkia*

specialist pollinators are absent, and overall bee abundance and diversity lower, outside the range limit, which could result in increased pollen limitation of reproduction in sites outside the current range limit (Moeller 2005; Moeller 2006; Moeller et al. 2012; Anderson et al. 2015).

Though almost every terrestrial plant spends its life embedded in soil, we have a very limited understanding of how variation in soil environments influence large scale distributions of plant species (but see Nuñez et al. 2009; Stanton-Geddes et al. 2012; Brown & Vellend 2014; Osborne et al. 2018). Soil abiotic properties are highly heterogeneous at both small and large scales, and soil microbial communities are hyperdiverse, with often strong effects on plant phenotypes (Bennett et al. 2009; Lau & Lennon 2012; Wagner et al. 2014; Keymer & Lankau 2017). If compatible microbial mutualists are absent, or novel pathogens present, outside a plant species' range limit, novel soil microbial assemblages could impair fitness outside that limit (Peay et al. 2010; Brown & Vellend, 2014; Lankau & Keymer, 2016). Variation in abiotic properties of soil, such as nutrient and organic matter content, across a species' distributional boundary could also influence the potential for population colonization outside the range. Though field experiments manipulating edaphic factors are logistically difficult (and thus rare), they are essential to quantify the contribution of spatial edaphic variation to plant distributional limits.

This dissertation is an attempt to untangle the complex environmental gradient that occurs across and beyond *C. x. xantiana*'s distribution, and evaluate the relative importance of precipitation, mammal herbivory, pollinator limitation, and biotic and abiotic edaphic factors in setting the subspecies' geographic range margin (Fig. I.1). In Chapter 1, I focus on fatal mammal herbivory and evaluate its potential as a range limiting factor, with a conceptual approach based on foundational range limits theory. I show that probability of herbivory increases sharply near and beyond *C. x. xantiana*'s range margin, that this interaction has large effects on population mean fitness at the transplant site beyond the range edge, and that susceptibility to herbivory is largely mediated by plant phenology. In Chapter 2, I follow up on these results with a large field experiment at multiple sites inside and outside the range, estimating the effects of

geography, source population, herbivory, and pollen limitation on lifetime fitness across two years. Protection from herbivory and supplementation of pollen increased plant fitness three to seven-fold outside the range margin, and there was only limited evidence of local adaptation of *C. x. xantiana* populations. Both of the transplant experiments reported in these chapters captured both a relatively wet and a relatively dry year, and showed that the effect of herbivory on population mean fitness differed across abiotic contexts — in dry years, precipitation limited fitness outside the range edge, but when *C. x. xantiana* was largely "released" from abiotic stress wet years, herbivory strongly limited population mean fitness.

Chapters 3 and 4 focus on belowground - aboveground interactions. In Chapter 3, I use greenhouse and field experiments to ask how spatial variation in soil microbial communities influences plant local adaptation and the potential for range expansion in *C. x. xantiana*. Microbial communities from one site inside the range positively affected components of fitness in both the greenhouse and field, especially near to and beyond the range margin, and there was no evidence of local adaptation to microbial communities among plant populations. In Chapter 4, I report on an intensive field experiment where I factorially manipulated complete (i.e., biotic and abiotic) edaphic environments (growing plants with soil sourced from either within or beyond their native range) and precipitation to quantify the relative effects of within-range soil and increased precipitation on *C. x. xantiana* fitness outside its range margin. Across two years, edaphic environment had large effects on plant lifetime fitness that were similar in magnitude to the effects of precipitation. Moreover, mean fitness of plants grown with within-range soil in the low-water treatment approximated that of plants grown with beyond-range soil in the high-water treatment.

Species' geographic distributions will often reflect adaptation to their *n*-dimensional niche, and consequently, maladaptation to environments falling outside the limits of that niche space along important environmental axes. Despite its hyperdimensionality, we often condense this niche hypervolume to two axes, precipitation and temperature. The series of experiments here provide ample evidence that non-climatic factors, particularly biotic interactions, can have very large effects on

lifetime fitness outside a species' range margin and influence the location of that margin. Furthermore, gradients in herbivory and pollinator availability exhibit nonlinear spatial patterns that recent theory suggests are especially important for generating stable range limits. Across complex environmental gradients, it will likely often be a combination of factors that constrains a species' distribution. On a fundamental level, uncovering the relative importance of, and interactions between, these environmental variables in determining fitness elucidates how ecological gradients, organismal traits, population demography, and adaptive evolution interact to produce species' range limits. Regarding predictions for the movement and fate of species under contemporary environmental change, these results highlight the need to go beyond climate and incorporate species interactions and adaptive capacity in predictive distribution models and management of species.

Chapter 1

Biotic interactions contribute to the geographic range limit of an annual plant: herbivory and phenology mediate fitness beyond a range margin

ABSTRACT

Species' geographic distributions have already shifted during the Anthropocene. However, we often do not know what aspects of the environment drive range dynamics, much less which traits mediate organisms' response to these environmental gradients. Most studies focus on possible climatic limits to species' distributions and have ignored the role of biotic interactions, despite theoretical support for their importance in setting distributional limits. We used field experiments and simulations to estimate contributions of mammalian herbivory to a range boundary in the Californian annual plant *Clarkia xantiana* ssp. *xantiana*. A steep gradient of increasing probability of herbivory occurred across the boundary, and a reanalysis of prior transplant experiments revealed that herbivory drove several-fold declines in lifetime fitness at and beyond the boundary. Simulations showed that populations could potentially persist beyond the range margin in the absence of herbivory. Using data from a narrowly sympatric subspecies, *C. x. parviflora*, we also showed that delayed phenology is strongly associated with *C. xantiana* ssp. *xantiana*'s susceptibility to herbivory and low fitness beyond its border. Overall, our results provide some of the most comprehensive evidence to date of how the interplay of demography, traits, and spatial gradients in species interactions can produce a geographic range limit, and lend empirical support to recent developments in range limits theory.

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INTRODUCTION

Understanding the causes of species' geographic range limits is a fundamental problem in ecology and evolution. For the vast majority of species, however, we still cannot answer why an organism occurs on one side of its range boundary and not the other (Gaston 2009). Pinpointing the underlying environmental drivers and demographic and genetic mechanisms restricting species distributions is of utmost importance for understanding species' responses to global change (Alexander et al. 2015; Ettinger and HilleRisLambers 2017), the spread of invasive species (Colautti et al. 2010), and the limits to natural selection (Antonovics 1976; Kawecki 2008).

Some species have simply not had time to colonize environmentally suitable areas (dispersal lag; Svenning et al. 2008; Alexander et al. 2017), and in other cases, abrupt dispersal barriers can prevent range expansion (Chardon et al. 2015; Weir et al. 2015). However, most species' borders occur along seemingly gradual environmental gradients (Kirkpatrick and Barton 1997; Sexton et al. 2009) and likely reflect underlying variation in the environment across the landscape and corresponding variation in adaptation. Species may be restricted to their current distribution simply because they are maladapted to the environment beyond their range boundary.

Several theoretical models address the apparent "failure" of natural selection to result in adaptation to novel environments outside a species' range (e.g., Kirkpatrick and Barton 1997; Case and Taper 2000; Polechová and Barton 2015). Population dynamics in these models are based upon the difference between a population's realized value of some important trait, and the optimal trait value dictated by the environment; this difference determines the degree of population maladaptation and population growth (Kirkpatrick and Barton 1997). A key factor in these models of range limits is the steepness of the environmental gradient along which populations must adapt. As gradients become steeper, adaptation to areas outside the current range becomes less likely due to high levels of maladaptation in colonists dispersing from the range edge; with shallow clines, adaptation and expansion of the range limit can proceed (Kirkpatrick and Barton 1997; Polechová and Barton 2015). Most models assume linear gradients in environmental variables, but non-linear gradients can be especially important in

generating distributional limits due to rapid change in optimal phenotype across space (Case and Taper 2000; Polechová and Barton 2015).

Given the central role of environmental gradients in structuring species' distributions, identifying important gradients is usually a first goal of range limit studies, with climatic variables being likely candidates. While climatic niche limits often do explain species' distributions (Lee-Yaw et al. 2016), it is increasingly recognized that biotic interactions can contribute to large scale distributional limits (Bruelheide and Scheidel 1999; Hochberg and Ives 1999; Case and Taper 2000; Briers 2003; Case et al. 2005; deRivera et al. 2005; Araújo and Luoto 2007; Holt & Barfield 2009; Gravel et al. 2011; Stanton-Geddes and Anderson 2011; Moeller et al. 2012; HilleRisLambers et al. 2013; Afkhami et al. 2014; Hargreaves et al. 2014; Louthan et al. 2015; Baer and Maron 2018). However, most evidence for biotic interactions influencing range limits is correlational, and there is a paucity of empirical studies that connect spatial gradients in biotic interactions to population demography and the geographic range limits of native species (Louthan et al. 2015).

Though correlative approaches such as species distribution models lend first insights into potential drivers of range limits, transplant experiments including sites outside the range limit are the only way to test range-boundary hypotheses directly (Hargreaves et al. 2014). When paired with field measurements of potentially important traits, transplant experiments can reveal trait-environment relationships that underlie geographic variation in performance (Hoffmann and Blows 1994; Angert et al. 2008; Sexton et al. 2009; Hargreaves et al. 2014). These trait-environment correlations can then be investigated further through direct manipulation of traits [e.g., production of a segregating F2 generation to generate phenotypic variation in traits of interest (e.g., Angert et al. 2008), or directly manipulating traits such as phenology (e.g., Griffith and Watson 2006)].

Investigating ecological causes of a species' distributional limit thus has three main components: characterizing environmental gradients, linking gradients to individual and population fitness, and determining the trait(s) mediating fitness responses. Studies rarely tackle these three points in concert (but see Angert et al. 2008), especially in regard

to biotic interactions. Here we investigate the role of an antagonistic interaction, fatal mammalian herbivory, in limiting the range of an annual plant, *Clarkia xantiana* ssp. x*antiana* (hereafter, *xantiana*). With two years of stem-translocation experiments, we show that this herbivory exhibits a steep, non-linear gradient across a major range boundary. Based on a two-year reciprocal transplant experiment across the same boundary (Geber and Eckhart 2005), we calculated the magnitude of mammalian herbivory over *xantiana*'s full lifespan, and used those estimates in simulations of herbivory's effects on population mean fitness. These simulations revealed large reductions in population growth rates due to herbivory at the range margin and outside the range, and showed that in the absence of herbivory, populations could potentially persist outside the range margin. Finally, we showed that susceptibility to herbivory is strongly associated with a specific plant trait, phenology, beyond the range margin.

METHODS

Study System

Clarkia xantiana comprises two annual subspecies, *C. x.* ssp. *xantiana* A. Gray and *C. x.* ssp. *parviflora* (Eastw.) Harlan Lewis and P.H. Raven (hereafter and above, *xantiana* and *parviflora*). Their combined range in the Southern Sierra Nevada foothills spans a complex west-to-east environmental gradient with *xantiana* found in the wetter, western region in oak woodlands, and *parviflora* found in the eastern region in arid scrub and pinyon-juniper woodland (Fig. 1.1; (Eckhart and Geber 1999).

The two taxa are in secondary contact (in a narrow zone of sympatry) after diverging ca. 65,000 years ago (Pettengill and Moeller 2012*a*, 2012*b*), and have differentiated most strongly in mating system and phenology (Eckhart and Geber 1999). *Parviflora* completes its life cycle more quickly than *xantiana*, which contributes to the near complete reproductive isolation between the subspecies (Briscoe Runquist et al. 2014). A reciprocal transplant experiment showed each subspecies to be strongly locally adapted to its own home range, and there was preliminary observation that herbivory by small mammals influenced *xantiana*'s performance beyond its range edge (Geber and Eckhart 2005). Mammalian herbivory occurs most often from two lagomorphs, the desert

cottontail (*Sylvilagus audubonii*) and the black-tailed jackrabbit (*Lepus californicus*), and less often from smaller rodents. These herbivores typically cause fatal herbivory, where the entire aboveground portion of a plant is removed (e.g. clipped at the stem base) and the plant does not resprout to set any seed.

We used two data sets in the analyses presented below. The more recent (2015-2016) uses stem translocation experiments to model fine-scale geographic trends in probability of fatal herbivory for *xantiana*, which allows us to link these results to new range-limits theory on geographic gradients in trait optima. To provide a uniquely comprehensive picture of how this biotic interaction affects fitness within and outside a range limit, we also analyze a previously published data set (transplant experiment years 1997-1999; Geber and Eckhart 2005) that includes information on lifetime fitness and herbivory at three sites: at *xantiana*'s range center, at the range edge, and beyond the range.

Quantifying gradients in herbivory across and beyond the range

To identify fine-scale spatial and temporal variation in plant-herbivore interactions, we performed a stem-translocation experiment across two years at 15 sites inside and outside *xantiana'*s range. Clipping living adult stems from natural populations to establish experimental arrays, we quantified herbivory while avoiding confounding spatial variation in genotype, plant size, or phenology found among *xantiana* populations. Experiments were conducted in or near natural *xantiana* and *parviflora* populations.

In 2015, we quantified broad-scale variation in herbivory across most of the west-east extent of *xantiana'*s range and beyond the range limit. We sourced *xantiana* stems from the center of the range, and within 6 km of the eastern edge; stems were collected from plants that were still green (i.e., with buds, flowers, and/or immature fruits). We placed stems at seven sites (two at range center, three at range edge, two that were 5 and 14 km beyond the eastern range limit; Fig. 1.1A). At each site we installed two transects of 24 stems, alternating central and edge genotypes, with stems placed 1 m apart. Plant stems were maintained in 13 cm florist picks filled with water and secured with an attached metal rod sunk into the ground (Appendix 1 Fig. A1 A,B). Plants maintained in

this way continued to open new flowers and set fruits after pollination (JW Benning, pers. obs.). To explore temporal variation in herbivory, we installed four temporal replicates of stems in May and June (approximately once per week from 24 May - 19 June) at each site. For each temporal replicate we scored stems for fatal herbivory (having no buds, flowers, or fruits remaining, usually because most of the stem was completely removed) five days after installation (Appendix 1 Fig. A1D). At the five sites within *xantiana*'s range, we also followed naturally occurring plants near experimental arrays to determine whether geographic patterns of herbivory on experimental plants mimicked that on natural plants (Appendix 1 A.1).

Our 2015 experiment showed that herbivory was low in the range center and much stronger at the range edge and beyond; however, the coarse geographic scale covered did not allow for a fine-scale characterization of the environmental gradient at the range limit. In 2016, we established experimental arrays in six sites near to or at the range limit, and five sites outside the range limit (Fig. 1.1A). As the 2015 experiment showed no effect of population source (central vs edge genotypes), plants used in 2016 were a mixture of genotypes from across the range. At each site we installed three transects of 10 stems placed 1 m apart. In an attempt to further mimic natural plant conditions, we placed 2016 stems in 50 mL conical tubes sunk completely into the ground (Appendix 1 Fig. A1B). We installed three temporal replicates of stems at each site and scored herbivory five days after installation. In 2016, wildfires destroyed the third round of experimental stems at three sites.

We used logistic regression to test the effects of easting (i.e., longitude), time (temporal replicate), and, in 2015, genotype source (central vs. edge), and all interactions, on the probability of herbivory. For both years, transect was included as a term nested within census date and easting position. Models were constructed using the glm function in R (R Core Team 2017), with binomial error distribution and logit link. We used BIC (Bayesian Information Criterion) scores to compare models with linear, linear plus quadratic, and linear plus quadratic plus cubic easting terms. We tested the significance of each term using Type II ANOVAs with likelihood ratio tests (car package, Fox and Weisberg 2011).

Quantifying the effects of herbivory on population fitness

We used data from a two-year reciprocal transplant experiment to ask how herbivory affects population fitness and the likelihood of population persistence across and beyond the range limit of *xantiana*. We compared our results for *xantiana* to those of *parviflora* as a means of identifying how trait differences between the two taxa may result in differing performance and susceptibility to herbivory. The majority (86%) of plants that suffered mammalian herbivory in the experiment set no seed (i.e., herbivory was fatal and lifetime fitness was zero); "herbivory" below refers only to these cases of fatal herbivory.

Reciprocal transplant In 1997-1999, we conducted a reciprocal transplant experiment to examine variation in phenotypic traits and lifetime fitness of both subspecies planted within and outside their respective ranges. In each year of this experiment, we planted 6 populations of *xantiana* and 12 populations of *parviflora* at one site within *xantiana*'s range center (but outside *parviflora*'s western range limit; Center), one site at *xantiana*'s range edge where it overlaps narrowly with *parviflora*'s range (Edge), and one site beyond the eastern *xantiana* range limit (but within *parviflora*'s distribution; Beyond-Edge; Fig. 1.1A). We planted seeds into 8,488 planting positions (eight seeds per position) arranged in 10 blocks per site in October and scored germination and survival monthly from January through July, culling to one seedling per position after germination. The experiment included a supplemental pollination treatment in a subset of blocks; the fitness analyses below exclude these blocks (as in Geber & Eckhart 2005). The two years of the experiment differed markedly in precipitation, and this led to strong differences in lifetime fitness estimates between years; hereafter and above, we refer to the two years of the experiment as "wet" (1997-1998) and "dry" (1998-1999). Full experimental details can be found elsewhere (Eckhart et al. 2004; Geber and Eckhart 2005).

Simulation of fitness in the absence of herbivory We took a post-hoc simulation approach to estimate mean population fitness at each site under two scenarios — no fatal herbivory and reduced fatal herbivory – and compare these fitness estimates to those derived from the observed data set. We first simulated a scenario where there was no

fatal mammalian herbivory during the two-year field experiment. In essence, we took the original experiment's data set and, for each plant that suffered fatal herbivory, estimated how many seeds it would have produced had it not been eaten. Predictive models were evaluated using R^2 statistics, Kolmogorov-Smirnov tests, and comparisons of predicted versus observed values (see Appendix 1 B for details on model construction and evaluation). We used these predictive models built with field data to produce lifetime fitness estimates for eaten plants that reflected all other environmental aspects of the sites, while "removing" herbivory. We simulated these data 100 times to allow for stochastic fluctuations in predicted fitness for these eaten plants (Appendix 1 B.2). As in Geber and Eckhart (2005), average lifetime fitness through female function (i.e., seeds produced per seed planted) for each planting position was calculated as the number of germinants times the product of predicted seed set and probability of reproduction (0 or 1).

After calculating predicted fitness values for eaten plants, we examined the extent to which average lifetime fitness would change at each site if there were no fatal mammalian herbivory. We estimated average lifetime fitness through female function (seeds produced per planted seed) for each subspecies at each site in both years. We used linear mixed models of lifetime fitness with site, year, and subspecies as fixed factors, and block (nested within site and year) and population (nested within subspecies) as random factors (as in Geber and Eckhart 2005). We built these models for each of the 100 simulated data sets; overall fitness estimates were averaged over the 100 model estimates. Comparison of the least-square means from models based on the original data (with herbivory) and this simulation (no herbivory) estimated the influence of herbivory on average lifetime fitness for each subspecies at each site.

Simulation of fitness beyond the range edge with reduced herbivory In the transplant experiment, herbivory rates beyond the range edge were ca. 100% higher than at the center and edge of the range (Results). Thus, we were also interested in simulating a more "moderate" scenario where herbivory was not completely absent, but rather, herbivory rates beyond the range edge were similar to rates within the range. Thus we used the same fitness simulations for eaten plants as above, but estimated mean fitness

for both subspecies under the scenario where herbivory rates in the Beyond-Edge site were the same as in the Edge site (i.e., a reduction instead of complete removal of herbivory; details in Appendix 1 B.3). The lifetime fitness estimates for each subspecies in the Beyond-Edge site for both years were averaged over the 100 simulations. Comparison of the predicted model means using the original data and this reduced herbivory simulation estimates the effect of increased herbivory rates outside the range limit on *xantiana* population persistence.

To what extent does plant phenology mediate susceptibility to herbivory?

We predicted that differences in development rate between *parviflora* and *xantiana* contributed to the former's escape from late season mammalian herbivory at the Edge and Beyond-Edge sites during the transplant experiment (Appendix 1 Table B1, Fig. B1), given observations that *parviflora* individuals are often dry and senescent when *xantiana* is still green and likely attractive to herbivores. Thus we tested whether plant phenology (as measured by flowering date) influenced a plant's probability of late season herbivory, using data from the transplant experiment. We were not interested in the date of flowering *per se*, but rather in using this as a proxy for a plant's developmental speed. Thus, we predicted date of flowering for plants that died before flowering (from herbivory or other factors), enabling us to "recover" this missing phenological information and make more robust estimates of model parameters (Appendix 1 C.1).

Due to the very low survivorship and low incidence of herbivory in the dry year, the analyses below are only for the wet year. We tested the effect of date of flowering, with plant size and block as covariates, on a plant's probability of fatal herbivory at each site using logistic regression with binomial error distribution and logit link. Because phenology is positively correlated with size in *C. xantiana* (Pearson's *r* of log(size) and date of flowering = 0.47), we included size (here, the largest size a plant achieved) as a covariate in the models to isolate the effects of phenology. Plant size was calculated as the product of plant leaf number and average leaf length. Since we were interested in the relationship between phenology and late season herbivory only, these analyses were restricted to plants that survived early season herbivory (i.e., were alive at the March

census); analyses including early season herbivory produced qualitatively similar results (Appendix 1 C.4). Since some plants for which we predicted flowering date died from factors other than herbivory (thereby precluding any later herbivory), these tests are somewhat conservative (i.e., some plants with predicted flowering dates were not eaten simply because they died before herbivores had the chance to eat them); in plots below we differentiate those plants that died from factors other than herbivory to assist in interpretation. We tested the significance of each term using Type II ANOVAs with likelihood ratio tests (car package, Fox and Weisberg 2011). We also ran these same models including subspecies as a term, to address potential confounding of phenology with other subspecies' differences (Appendix 1 C.3).

We estimated optimal flowering dates at each site by fitting a loess smoother to the function *log(fitness) ~ flowering date* to find the flowering date at which fitness was maximized. We included both subspecies to increase the phenological range over which we could evaluate fitness, and included all plants that were alive at the March census (details in Appendix 1 C.7).

RESULTS

Stem translocation experiment

Herbivore pressure increases at and beyond the range limit

In 2015, the probability of fatal herbivory on translocated *xantiana* was close to zero at the range center and increased sharply near the range limit, exceeding 0.75 beyond the range limit (Fig. 1.2A). The pattern of herbivory was best fit with the logistic model including longitude (easting) as a linear term (BIC: 1324; N = 1278; Nagelkerke's $R^2 = 0.49$; Appendix 1 Table A1). Overall, the odds of a plant being eaten increased 9% for every kilometer eastward ($\chi^2 = 498.2$, $P < 0.001$), with the gradient in probability of herbivory becoming very steep near the range limit. For example, in the last census round, the probability of herbivory increased from 0.01 at the most central site to 0.13 10 km east of that site, but over the next 10 km eastward, increased to 0.7 approximately at the range limit. There was also a significant interaction of longitude with time ($\chi^2 = 41.5$, $P < 0.001$), with probability of herbivory increasing as the season progressed at the range-

edge and beyond-range sites, but not in the range center (Fig. 1.2A). Genotype (plants sourced from the center vs. edge of the range) had no effect on probability of herbivory (χ^2 = 0.36, P = 0.5). Within *xantiana*'s range, herbivory on translocated stems generally matched that on natural plants, with rates at four of five sites differing by less than 5%; translocated stems experienced much more herbivory at one near-edge site but removing this site did not qualitatively affect the modeled gradient in probability of herbivory (see Appendix 1 A.1).

In 2016 the pattern of herbivory was best fit with a logistic model including longitude as linear and quadratic terms (BIC: 696; N = 561; Nagelkerke's R^2 = 0.33; Appendix 1 Table A1). Probability of herbivory was low 10 km inside the range limit (ca. 0.07), increased toward the range limit to a maximum of ca. 0.62 eight kilometers beyond the limit, and decreased further east (Fig. 1.2B). Probability of herbivory also increased from the first census round to later rounds (χ^2 = 86.3, P = 0.002), though there was no significant interaction of time with easting as in 2015.

Transplant experiment

Herbivory threatens population persistence beyond the range limit

In the first, wetter year of the experiment, *xantiana* and *parviflora* suffered equal rates of herbivory (15% of germinated plants eaten) at the Center site, but *xantiana* experienced higher herbivory further east (*xantiana*: 34% and *parviflora*: 8% at the Edge site; *xantiana*: 54% and *parviflora*: 19% at the Beyond-Edge site; Appendix 1 Table B1). In the second, dry year, herbivory was very low throughout (1-5%; Appendix 1 Table B1).

When we simulated a scenario with no fatal herbivory, effects on fitness were observed in the wet year but not the dry year, when plant survival and performance were low and herbivory rare. In the wet year, removal of herbivory had the largest effect on lifetime fitness for *xantiana* in the Edge and Beyond-Edge sites, increasing lifetime fitness two and six fold, respectively, but *xantiana* mean fitness increased only 40% at the Center site (Fig. 1.3; Appendix 1 Table B4). Interestingly, removing herbivory beyond the range edge brought estimates of *xantiana* average lifetime fitness to 1 (i.e.,

population replacement). Removing herbivory also increased estimates of *parviflora* fitness at the Edge and Beyond-Edge sites, but the effects were much smaller (24% and 107% increases, respectively; Appendix 1 Table B4, Fig. B3).

When we simulated a scenario where herbivory was reduced in the Beyond-Edge site to levels observed at the Edge site, *parviflora* and *xantiana* experienced increases in lifetime fitness estimates in the wet year but not in the dry year (Fig. 1.3; Appendix 1 Table B4). In the wet year, average lifetime fitness for *parviflora* increased 50% to 3.63 (Appendix 1 Fig. B3) and for *xantiana* increased 300% to 0.60 (Fig. 1.3).

Delayed phenology is associated with fatal herbivory

Logistic regression showed that phenology was associated with probability of herbivory on *xantiana* and *parviflora* at all sites, and especially strongly at the Edge and Beyond-Edge sites (Fig. 1.4). For each day delay in flowering, a plant's odds of herbivory in the range Center, Edge, and Beyond-Edge sites increased significantly by 2% ($\chi^2 = 3.9$, $P < 0.05$), 5% ($\chi^2 = 53.8$, $P < 0.001$), and 14% ($\chi^2 = 118.0$, $P < 0.001$), respectively (Appendix 1 Table C3). At the Edge and Beyond-Edge sites, larger plants were more likely to be eaten ($P < 0.002$); whereas in the Center site, smaller plants were more likely eaten ($P < 0.001$). Block effects at all sites ($P < 0.001$) indicated fine-scale spatial heterogeneity in herbivory. Differentiation in phenology between the subspecies is illustrated in Figure 1.4, where *parviflora*'s earlier phenology is apparent. This difference is associated with a marked subspecies difference in susceptibility to fatal herbivory at the Edge and Beyond-Edge sites. When we included subspecies as a term in the models to account for potential confounding of phenology with some other subspecies' difference, flowering date was still highly significant ($P < 0.001$) beyond the range edge, but was not at the Center or Edge sites (Appendix 1 C.3)

Comparing optimal versus realized mean flowering dates showed that *xantiana* was far from the phenological optimum (ca. 18 days later) outside its range, but was within ca. 4 days of optima at Center and Edge sites (Fig. 1.4).

DISCUSSION

Recent reviews of transplant experiments support the idea that species' geographic range limits often reflect niche limits (Hargreaves et al. 2014; Lee-Yaw et al. 2016). But given the demonstrated power of natural selection to produce adaptations to novel environments, what prevents range expansion via sequential adaptation of marginal populations? The vast majority of work on geographic range limits has focused on gradients in abiotic variables, mainly temperature and precipitation. However, the field is increasingly calling for tests of how biotic interactions can modulate range boundaries, given experimental (e.g., Moeller et al. 2012; HilleRisLambers et al. 2013; Afkhami et al. 2014), theoretical (e.g., Hochberg and Ives 1999; Case and Taper 2000; Case et al. 2005; Gravel et al. 2011), and indirect / correlational (e.g., Araújo and Luoto 2007, Ettinger et al. 2011; Pigot and Tobias 2013; Scully et al. 2018) evidence for the influence of species' interactions on large-scale distributions. Here we showed that an antagonistic biotic interaction, mammalian herbivory, has large effects on population mean lifetime fitness at and beyond the subspecies' geographic range limit, and that probability of herbivory exhibits a steep gradient across the range of *C. xantiana*. We then showed that a specific plant trait, phenology, is strongly associated with probability of herbivory at and outside the range limit. Together, this set of results provides some of the strongest evidence to date that biotic interactions can play a pivotal role in determining the location of a geographic range limit.

Transplant and translocation experiments

Our simulations using the transplant data set showed that at range center, removal of herbivory had minor effects on *xantiana* lifetime fitness, but at and beyond the range edge, a complete absence of herbivory increased *xantiana* lifetime fitness two- and five-fold, respectively. For annual plants like *xantiana*, population mean lifetime fitness approximates population growth rate (λ). Interestingly, these simulations imply that in the absence of herbivory, *xantiana* population growth at the range edge could be double that at range center, and that populations beyond the range edge could potentially replace themselves. This highlights how a biotic interaction can influence population

demography at a species' range edge, and potentially emigration and colonization outside the range limit.

When we simulated reduced herbivory outside the range (instead of complete removal), *xantiana* mean lifetime fitness increased 300% relative to field data in the wet year, to $\lambda = 0.60$. Though this is still below levels needed for population replacement, adaptive evolution beyond the range margin could potentially raise population mean fitness above replacement, given adequate heritable variation in ecologically important traits. There is evidence of substantial genetic variance for fitness in *xantiana* planted beyond its range limit (Moeller et al., unpubl. data), which could allow population mean fitness to evolve and populations to "escape" extirpation (Fisher 1930; Gomulkiewicz and Shaw 2013).

The most direct test of the influence of herbivory on population fitness would be to manipulate access by herbivores with caging in the field. Here we took an alternative, post-hoc simulation approach that allowed us to estimate mean population fitness at each site under two scenarios — no fatal herbivory and reduced herbivory – and compare these fitness estimates to those derived from the observed data set. Of course, our fitness predictions for eaten plants cannot be perfect reflections of what would have happened *sans* herbivory in the field. However, simulating fitness values across multiple instantiations of the experiment *in silico* allowed for stochasticity in the prediction process (see Appendix 1 B), and provided a conceptually rigorous approximation of population fitness under different scenarios.

Our stem translocation experiments showed that herbivory exhibits a steep gradient across and beyond *xantiana*'s range, with a sharp increase in probability of herbivory near the eastern range margin. For example, during the last stem census in 2015, *xantiana* at the center of the range had less than a 5% chance of fatal herbivory, while only 8 km outside its range limit, the probability of herbivory was over 15 fold higher (95%). This spatial pattern is in accord with predictions from range limit models that the steepness of relevant environmental gradients is key to generating species' distributional boundaries (Kirkpatrick and Barton 1997; Polechová and Barton 2015).

Phenology and herbivory

These above findings speak to the proximate, ecological causes of *xantiana*'s range limit, but the ultimate cause of a range limited by adaptation is genetic limits on trait evolution. We rarely know which traits would need to evolve to allow range expansion (but see Hoffmann et al. 2003; Griffith and Watson 2006; Angert et al. 2008; Colautti et al. 2010). In this study, we were able to use differentiated sister taxa to ask how a specific trait, phenology, influenced probability of herbivory at multiple sites. While phenology had little effect at range center, the difference in phenology between the two subspecies beyond the range limit was associated with large differences in susceptibility to fatal herbivory. It is certainly possible that other, unknown traits differing between the subspecies (e.g., defensive compounds) could contribute to *xantiana*'s increased probability of herbivory, though even when we include subspecies as a term in our models of herbivory given phenology, phenology remains a significant predictor outside the range edge (see Appendix 1 C.3). The link between phenology and probability of herbivory is additionally supported by the significant effect of time (i.e., early to late growing season) in our statistical models of the stem translocation results — plants were more likely to be eaten as the season progressed (except for sites near the range center, where probability of herbivory was consistently near zero). This approach eliminated potential confounding of phenology with other subspecies' traits, as the translocation experiment only used *xantiana*, and allowed us to ask how the probability of herbivory on green, non-senescent plants varied across the growing seasons of both subspecies (i.e., early: *parviflora*; late: *xantiana*).

Phenology has been shown to be a key range-limiting trait in other plant species, though usually in the context of abiotic latitudinal range limits (Griffith and Watson 2006; Colautti et al. 2010). For *xantiana*, it seems phenology would have to evolve to enable eastward range expansion. Indeed, phenology *did* evolve in ancestral *xantiana* populations that diverged in allopatry to become *parviflora*, which later expanded in range such that it is now in secondary contact with *xantiana* (Pettengill and Moeller 2012b). Thus the question becomes, what is now preventing adaptive evolution at *xantiana*'s range limit?

Linking to theory Recent theoretical work (Polechová and Barton 2015; Polechová 2018) showed that in models including genetic drift, a range margin can form via two (non-mutually-exclusive) mechanisms: a steepening (i.e., non-linear) environmental gradient driving increasing maladaptation, or a decrease in carrying capacity across space leading to an increased influence of drift on population genetic variance. Both of these factors could be at play for *xantiana*. In these models, a steepening environmental gradient creates a sharp range margin near the environmental "inflection point." This is due to drift eroding genetic variance needed to adapt to a quickly changing trait optimum as small, colonizing populations encounter new environments to which they are very poorly adapted. The result is that population trait means closely track trait optima along most of the environmental gradient, but fail to do so when this gradient suddenly steepens, like the gradient in probability of herbivory does near *xantiana*'s range limit. This increased mismatch between optimal and observed trait values drives demographically unsustainable declines in population mean fitness, which is in agreement with our empirical estimates of the difference between observed and optimal flowering dates outside the range margin (ca. 18 days), compared to within *xantiana*'s natural range (ca. 4 days). Increased herbivore pressure could also impose an extrinsic limit on *xantiana*'s carrying capacity outside its range edge, depressing population sizes so as to make any populations able to colonize outside the range limit more susceptible to drift eroding potentially adaptive genetic variance. The concordance of observed patterns in environmental variation and *xantiana*'s distribution with model predictions provides empirical support for recent range limit models (Polechová 2018).

Why does herbivory vary across space?

Geographic variation in herbivory across *xantiana*'s range could be explained by two phenomena. First, the herbivore community likely changes across *xantiana*'s range. Our field observations and surveys using motion-triggered cameras (2015 and 2016) suggest that two lagomorph herbivores often eat plants outside the range (desert cottontail and black-tailed jackrabbit) whereas only the desert cottontail is common in the center of *xantiana*'s range (Appendix 1 Fig. A1C). Habitat descriptions support these observations,

reporting that the black-tailed jackrabbit is more common in arid, open scrubland typical of sites at and outside *xantiana*'s eastern range boundary (Arias-Del Razo et al. 2012). If there is increased herbivore pressure near *xantiana*'s range limit due to an additional lagomorph species, this could translate into higher herbivory rates on *xantiana* planted at and outside its range limit.

A second, non-mutually-exclusive hypothesis is based on decreases in primary productivity, especially of herbaceous plants, across the west-to-east gradient (Fig. 1.1A). The availability of more forage at *xantiana*'s range center may dilute herbivore pressure on *xantiana*. In contrast, in the more arid east where *parviflora*'s distribution is centered, *xantiana* may be increasingly attractive to herbivores due to limited forage and its late completion of development compared to co-occurring forbs. Field observations suggest this pattern arises because *parviflora* is less palatable forage by the peak of late season herbivory, whereas *xantiana* is still green and flowering. For example, during transplant experiments, *xantiana* was often the only herbaceous vegetation still green by early June, when surrounding ephemerals had already senesced.

Temporal variability and abiotic × biotic interactions

Another important takeaway from this study is that environmental constraints on species' ranges need not be static across time. In the dry year, fitness was limited outside the range (and everywhere) by low precipitation. In the wet year, the geographic gradient in aridity led to relatively fewer germinants in the Beyond-Edge site, but our simulations showed that the population may have been able to persist in the absence of herbivory. This sort of temporal variation in selection could prevent or slow changes in the frequency of beneficial alleles (Kirkpatrick and Peischl 2013). This highlights how temporal variability can alter selective environments and create "moving targets" for evolution at range edges (Hao et al. 2015), and echoes the recommendation of Hargreaves et al. (2014) that transplant experiments should occur over multiple years to capture as much temporal variation as possible.

In the wet year of the transplant experiment, the number of plants eaten by herbivores was 25 percent higher in the Beyond-Edge site than the Center site (251 and

203, respectively). However, the *proportion* of plants eaten, given the number of germinants, was double beyond the edge (31 vs. 15 percent), due to the lower number of germinants beyond the range edge. Thus, the effect of herbivory on population growth was compounded via other, likely abiotic, factors (precipitation). This demonstrates how multiple environmental factors can interact to influence the distribution of a species.

The multivariate nature of range expansion

Thus far we have considered phenology in isolation, but range-edge *xantiana* populations will likely have to evolve multiple traits to colonize outside their range boundary (Antonovics 1976). To colonize areas outside its eastern range limit, where its sister taxon occurs, *xantiana* would likely need to adapt to not only increased herbivore pressure, but lower and increased variation in precipitation, and less abundant pollinator communities. For example, due to the low abundance of pollinators [especially *Clarkia* specialist bees (Moeller 2006)] and high pollen limitation at and beyond its range edge (Moeller et al. 2012), *xantiana* would need to evolve a higher selfing rate for reproductive assurance. Similarly, given the increased temporal variation in rainfall in the east, increased seed dormancy would likely be advantageous outside the range limit (Eckhart et al. 2011). Thus, colonization of habitat beyond *xantiana*'s current range margin would likely require evolution of multiple ecologically-important traits involving many genetic loci, which could slow or prevent adaptive evolution at the range edge (Antonovics 1976; Duputié et al. 2012). The original divergence of *parviflora* from *xantiana* may have been aided by relatively shallow environmental gradients (see 'Linking to theory' above), or the opportunity for sequential adaptation in relevant traits as opposed to a sudden, concurrent shift in optima for multiple traits.

Generality of a generalist predator enforcing range limits

Given the strong effects of herbivory on individual plant fitness, population growth, and local and elevational distributions (Louda 1982; Quinn 1986; Bruelheide and Scheidel 1999; Fine et al. 2004), it is surprising that only one recent study has examined herbivory's role in modulating plant species' geographic ranges (Baer and Maron 2018).

To our knowledge, ours is the first study to explore the effects of herbivory on a geographic range limit using experimental transplants beyond the range boundary, which is optimal for the testing of range limit hypotheses (Hargreaves et al. 2014). Case et al. (2005) pointed out that, theoretically, polyphagous predators can easily enforce geographic range limits of prey species, especially when two prey species are differentially susceptible to predation over a spatial gradient. This is the pattern we see in *C. xantiana*, but should we expect that generalist herbivores often regulate geographic distributions of plant species? Rapid phenology is commonly observed in arid systems, and this has long been presumed to be due to selection to escape the late season drought and unpredictable hydric environments of arid areas (Aronson et al. 1992; Thuiller et al. 2004; Levin 2006; Volis 2007). "Phenological escape" from insect herbivory has been shown for multiple plant taxa (Pilson 2000; Krimmel and Pearse 2016; Mlynarek et al. 2017), but mammalian herbivore control on plant phenology and distributions in arid environments remains relatively unexplored.

Studies often focus on climatic control of geographic range limits, but given the intricate web of interspecific interactions in which every organism participates, we cannot ignore the potential role of biotic factors in structuring large-scale distributions. Combining multiple lines of evidence to link environmental variation, traits, and fitness, our study demonstrates how biotic interactions can generate adaptive hurdles for important traits and contribute to the formation of species' range limits.

Chapter 2

Maladaptation beyond a geographic range limit driven by antagonistic and mutualistic biotic interactions across an abiotic gradient

ABSTRACT

Species' geographic range limits often result from maladaptation to the novel environments beyond the range margin. However, we rarely know which aspects of the *n*-dimensional environment are driving this maladaptation. Especially of interest is the influence of abiotic versus biotic factors in delimiting species' distributions. We conducted a two-year reciprocal transplant experiment involving manipulations of the biotic environment to explore how spatio-temporal gradients in precipitation, fatal mammalian herbivory, and pollination affected lifetime fitness within and beyond the range of the California annual plant, *Clarkia xantiana* ssp. *xantiana*. In the first, drier year of the experiment, fitness outside the range edge was limited mainly by low precipitation, and there was some evidence for local adaptation within the range. In the second, wetter year, we did not observe abiotic limitations to plant fitness outside the range; instead biotic interactions, especially herbivory, limited fitness outside the range. Together, protection from herbivory and supplementation of pollen resulted in 3-7 fold increases in lifetime fitness outside the range margin in the abiotically benign year. Overall, our work demonstrates the importance of biotic interactions, particularly as they interact with the abiotic environment, in determining fitness beyond geographic range boundaries.

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INTRODUCTION

"It is undoubtedly always a combination of factors which accounts for an animal's geographic range in all parts of the periphery of that range. It is most certainly never one factor alone."

Joseph Grinnell, 1917

The distributions of species are determined largely by their environmental tolerances. Though historical contingencies and dispersal dynamics certainly influence these distributions, it is generally proposed that, for most species at broad scales, populations occur where the environment is suitable for them to persist (Darwin 1859; Grinnell 1917; MacArthur 1972). In these cases, somewhat tautologically, species are restricted to their realized niches (*sensu* Hutchinson 1957) — populations do not persist beyond their niche limits, which in physical space are realized as geographic range limits. This environmental filtering of organisms leads to spatial patterns of occurrence that form the basis of many ecological and evolutionary questions. Recent studies suggest that for many species, geographic range limits do reflect niche limits (Hargreaves et al. 2014; Lee-Yaw et al. 2016), whereas for others range limits are influenced by dispersal lag (e.g., Svenning et al. 2008; Alexander et al. 2018). However, the hyperdimensionality of these niches (Hutchinson 1957) means that, for most organisms, we do not know what aspects of the environment are important in setting these range limits (Grinnell 1917; Gaston 2009).

By far the most examined niche axes in regard to range limits are climatic variables such as temperature and precipitation. Partly due to their ease of measurement, and partly due to anticipated climatic changes, these abiotic variables have been at the forefront of most research on contemporary and future range limits (Sexton et al. 2009; Nadeau et al. 2017). The literature is rife with predictions for future species distributions based on shifting temperature isotherms, and indeed, there is good evidence that many species have already shifted up in latitude or elevation with recent warming (Parmesan and Yohe 2003; Chen et al. 2011; Rumpf et al. 2018). But important to note is the large variation in responses among taxa. For example, Chen et al (2011) found that 22% of the

species they examined underwent range shifts in the direction opposite that expected from climatic trends (e.g., downslope in spite of warming). Similarly, in a survey of plants in the European Alps, Rumpf et al (2018) found that nearly half of the species had at least one range attribute (range center, leading edge, or rear edge) that shifted downslope. Clearly climatic niches are not the whole story.

And we shouldn't expect them to be, given the myriad interactions every organism has with other species, and the wide-ranging consequences these biotic interactions have on the ecology and evolution of populations (Bridle and Vines 2007; Louthan et al. 2015; Urban et al. 2016). As far back as Darwin (1859), it was proposed that biotic interactions could set geographic range limits, and this idea is well supported by theory (Hochberg and Ives 1999; Case and Taper 2000; Gravel et al. 2011). Empirical evidence, especially experimental tests, are relatively scarce, but studies have supported the notion that mutualists (Moeller et al. 2012; Afkhami et al. 2014), competitors (Bullock et al. 2000; Ettinger and HilleRisLambers 2017), and predators (Bruelheide and Scheidel 1999; deRivera et al. 2005; Baer and Maron 2018; Benning et al. 2019) can influence the location of geographic range limits.

Despite its long history, the majority of work on range limits has been correlational — i.e., relating species occurrences to environmental variables (usually climatic) across the landscape. This approach is the foundation of modern descriptive and predictive species distribution models (SDMs). However, correlational approaches are confounded by the fact that many aspects of the environment covary across the landscape, and that the spatial autocorrelation of species distributions will rarely fail to correlate with some similarly spatially autocorrelated environmental variable (see Fourcade et al. 2018). By contrast, transplant experiments can offer much deeper insights into the causes of both local and/or elevational (e.g., Bruelheide and Scheidel 1999; Angert and Schemske 2005; Angert et al. 2008; Emery et al. 2009; O'Brien et al. 2017) and geographic range limits (e.g., Levin and Clay 1984; Geber and Eckhart 2005; Griffith and Watson 2006; Samis and Eckert 2009). They can directly test whether a range is limited by maladaptation, as opposed to failure to disperse. If paired with experimental manipulations of putatively important environmental variables, these experiments can

also isolate factors constraining range expansion (e.g., Griffith and Watson 2006; Anderson et al. 2015). Due to logistical complexity, manipulative transplant experiments are rare, but they are essential to untangle covarying environmental gradients and determine their relative importance in setting distributions. Range limit experiments ideally 1) incorporate multiple sites within and outside the range limit, 2) estimate lifetime fitness of the focal species, and 3) manipulate putatively important environmental factors (Hargreaves et al. 2014). These experimental approaches are also ideally coupled with a historical perspective on range dynamics offered by population genetic approaches (Moeller et al. 2011).

When researchers do examine biotic constraints on species' distributions, they most often find evidence supporting the role of such interactions in contributing to geographic range limits. However, most of these studies are, again, correlational (Sexton et al. 2009; Louthan et al. 2015), finding negative correlations between the density of a focal species and some putative competitor or predator. Of course, if the distributions of these two species are also associated with adaptation to particular abiotic conditions that vary across the landscape, the emergent patterns could look very much like those predicted by a limiting effect of biotic interactions (but see Aragón & Sánchez-Fernández 2013). *Lack* of evidence for abiotic controls is also sometimes presented as evidence for biotic controls on range limits (e.g Ettinger et al. 2011; O'Brien et al. 2017). Manipulative experiments are the most direct tests of causal relationships between distributions and species interactions (facilitation, competition, etc.). There are very few studies that both manipulate a biotic interaction in a transplant experiment within and beyond a geographic range limit, and calculate the interaction's direct effect on components of lifetime fitness (but see Stanton-Geddes et al. 2012; Anderson et al. 2015; Katz & Ibáñez 2017).

We investigated the influence of two biotic interactions on lifetime fitness in a California annual plant, *Clarkia xantiana* ssp. *xantiana* (Onagraceae), within and outside its geographic range limit. As is likely to be the case with most species, *C. x. xantiana* is distributed across a complex environmental gradient comprising many covarying abiotic and biotic environmental variables. Both probability of herbivory and pollinator

availability change across the subspecies' range (increase and decrease, respectively), and prior work has demonstrated that spatial variation in these interactions can have large fitness consequences for *xantiana* (Anderson et al. 2015; Benning et al. 2019). We used a manipulative transplant experiment across two years and at multiple sites within and outside the range to address three sets of questions:

1. How does lifetime fitness vary from the center to edge of the range and in multiple sites beyond the edge?
2. To what extent is there local adaptation in different parts of the range? Do source populations differ in lifetime fitness outside the range edge?
3. How do interactions with mammalian herbivores vary across the range and beyond? To what extent does amelioration of herbivory and pollen limitation of reproduction increase fitness, alter patterns of local adaptation, and affect the likelihood of population persistence beyond the range edge?

METHODS

Study System

Clarkia xantiana ssp. *xantiana* A. Gray (hereafter, *xantiana*) is a winter annual native to the Southern Sierra Nevada foothills of California (USA) (Eckhart and Geber 1999). *Xantiana* is distributed across a complex west-to-east environmental gradient, with western and central *xantiana* populations found primarily on steep, sandy slopes in relatively mesic oak woodlands of the Kern River Canyon, and eastern edge populations found in drier pine woodlands (Fig. 2.1; Eckhart et al. 2011; Gould et al. 2014). Most populations, including all used in this study, occur on sandy, fast-draining soils derived from granitic rock (Eckhart et al. 2010). The eastern range edge is stark (Fig. 2.1) and extensive searching over the past 20+ years has uncovered no *xantiana* populations beyond this limit.

Xantiana is distributed across an aridity gradient (with precipitation lower and more variable toward and outside its eastern range edge) that contributes to reduced performance at the range edge and beyond (Eckhart et al. 2010, 2011). In contrast to most study systems, the influence of biotic interactions on *xantiana*'s distribution have

received a relatively large amount of attention in several within and beyond-range experiments. These studies have shown that mutualistic interactions with pollinators are weaker at and beyond the range limit, resulting in greater pollen limitation of reproduction (Moeller et al. 2012; Anderson et al. 2015). By contrast, antagonistic interactions with mammalian herbivores (primarily lagomorphs: *Sylvilagus audubonii* and *Lepus californicus*) are stronger at and beyond the range limit (Benning et al. 2019). The current study builds on these prior efforts in three important ways. First, prior transplant studies occurred at relatively few sites, whereas the current study involves three sites within the range, and three beyond. Second, while Benning et al. (2019) used a *post-hoc* simulation approach to estimate the effect of mammal herbivory on fitness in a previous transplant experiment, here we experimentally manipulate the presence versus absence of herbivores *in situ*. Third, the joint effects of gradients in the two key biotic interactions (pollinators and herbivores) on lifetime fitness, and their interactions with abiotic gradients, have not been estimated before this study.

Reciprocal transplant

To estimate the effects of multiple biotic interactions, geography, and source population on *xantiana* lifetime fitness, we planted seeds from three *xantiana* populations into six sites spanning from the center to 22 km outside the natural distribution of *xantiana*. The six sites were at *xantiana*'s range center (Center), between the center and range edge (Intermediate), eastern range edge (Edge), 5 km outside the eastern range edge (Just Beyond), 14 km outside the range edge (Beyond), and 22 km outside the range edge (Far Beyond) (Fig. 2.1; Appendix 2 SI.1). The three sites within the range contain natural *xantiana* populations, and the three sites outside contain its sister subspecies, *parviflora*. Sites containing *C. x. parviflora* are a good approximation of "suitable" habitat outside the eastern range edge of *C. x. xantiana*, given that both subspecies occupy similar niches and sites in the region where they are sympatric. The experiment was conducted across two growing seasons (2015-2016 and 2016-2017; hereafter, year 1 and year 2).

Xantiana seeds were sourced from the Center and Edge sites in year 1, and Center, Edge, and Intermediate sites in year 2. We did not include the Intermediate site in year 1 due to a seed collection error. In year 1, seeds used for planting were all collected from the field (30 to 70 maternal families per population). Due to drought and thus low site productivity in 2016, we generated seed for year 2 planting in the greenhouse by crossing among 26 to 30 maternal families per population. No crosses occurred within maternal families. Within source populations, we pooled seeds from all maternal families before planting.

At each of the six sites, we installed 120 plastic grids arranged into six blocks (20 grids per block) set into the natural vegetation matrix (N = 720 grids total). Grids were cut from white plastic diffusion screens (used for fluorescent light fixtures) and set onto the soil surface after scraping away the top ca. 1 cm of soil underneath to avoid contamination from the local *C. xantiana* seed bank, and filled with soil dug from ca. 20 cm below the surface (Fig. 2.1b). Grids comprised a 7 x 7 matrix of 3 cm x 3 cm cells (with ca. 2 cm high walls); only the inner 36 cells were planted to avoid potential edge effects. These grids allowed us to follow individual seeds while maintaining a natural growing environment for the experimental plants. We did not weed or otherwise alter natural vegetation around the grids. Because the grids are enmeshed in the surrounding matrix of soil and vegetation, and are almost level with the soil surface, they are unlikely to influence rates of herbivory. Incidental germination (i.e., *xantiana* germination in empty cells) was very low – e.g., at the Center site, where the *xantiana* seed bank is likely largest, 8 out of 1,920 empty cells had a germinant in year 1 (0.4%); any incidental germinants were removed from grids.

The source populations were randomly assigned to cells within grids using three randomized planting schemes (5 cells per source population per grid in year 1, and 4 cells per source population per grid in year 2); each grid was randomly assigned to a scheme. Two seeds were planted per cell in October of each year (in year 2, all seeds were planted into empty cells that were not planted into in year 1). Thus, in year 2, the experiment included newly planted seeds as well as any seeds that were planted in year 1 and did not germinate. We account for these different seed cohorts in our analyses.

We visited sites in February and March to score germination and early season survival, respectively, and monitored late season survival, mammal herbivory, and total seed set May through June in each year. If there were two germinants in one cell, we randomly culled one germinant to maintain natural plant densities. We estimated seed set in each collected fruit using a linear model that predicted fruit seed set as a function of individual fruit weight (SI.2). A plant's lifetime fitness is equal to the sum total seeds contained in all of its fruits.

We had access to precipitation monitoring stations (HOBO Onset) at four of the six transplant sites (Center, Intermediate, Just Beyond, and Beyond), which are part of a long-term study of *C. xantiana* population dynamics. For the Edge and Far Beyond sites, we used nearby weather stations (4 and 13 km from site, respectively) to estimate precipitation during transplant years. In order to interpret annual precipitation patterns relative to long-term trends, we used the PRISM climate dataset (PRISM Climate Group) to obtain interpolated estimates of monthly precipitation data for each site for years 1990 - 2017, at 4 km grid cell resolution (Appendix 2 Table S1).

Herbivore exclusion

In March of 2016 and 2017, half of the grids in each block were surrounded by open-topped 0.6 m high herbivore exclosures made from 1.3 cm galvanized steel mesh (Fig. 2.1c), unless the grid contained no plants. Grids were randomly assigned exclosure treatments, and we alternated exclosure grids between the two years (i.e., no grid was caged or uncaged for both years). Toward the end of the growing season in each year, we also attached tops to these exclosures to prevent rodents from breaching the cages.

The experiment also included a soil manipulation treatment that was fully crossed with the caging treatment, where we filled grids with soil from one of four source sites (local, Center, Intermediate, or Beyond) prior to planting. Results of these soil manipulations will be reported in a later manuscript; for the current analyses, all effects of biotic treatments, site, and source population are averaged over these soil microbial manipulations.

We found no evidence that caging itself affected plant growth (Appendix 2 SI.3, Table S2). We do note that, simply by chance, there were differences in germination among grids in caged and uncaged treatments at some sites (Table 2.3), even though grids were not caged until months after germination. The only instance where this could potentially confound interpretation of our results is at the Far Beyond site, where caged seeds showed slightly higher germination rates than uncaged seeds (0.11 vs. 0.09, respectively). Thus, the effect of caging at the Far Beyond site should be interpreted with a modicum of reserve.

Pollen limitation of reproduction

In year 2, we supplemented pollen on a subset of plants at all sites to estimate pollen limitation of reproduction across and beyond *xantiana*'s range. We performed the supplementation experiment on all plants that had at least two flowers, including one with a stigma that was receptive during our site visits. For each plant, we selected two flowers to serve as supplemented and control flowers (on some large plants, we selected an additional pair of supplemented and control flowers, but 94% of plants had only one supplemental / control pair). The control flower was either directly above or below the supplemented flower; relative orientation of the pair was haphazardly rotated among plants. Prior research on *C. xantiana* has shown that resource reallocation does not occur among neighboring fruits and therefore does not bias estimates of pollen limitation (Briscoe Runquist and Moeller 2013). If multiple flowers had receptive stigmas, we haphazardly chose one for supplementation, avoiding flowers at the ends of branches. We collected pollen from a natural *xantiana* population near the Edge site and applied pollen across the entire receptive stigmatic surface of each supplemented flower. Fruits were collected at maturity and all seeds counted.

We also quantified pollinator visitation by conducting pollinator observations at all sites during the flowering period. All flower visitors were bees, particularly *Clarkia* specialists inside the range (Moeller 2005; Moeller 2006). Flowers on subsets of experimental plants were watched for ten-minute periods and all bee visitors were recorded (average of 43 observation periods in each region; average of 13 flowers

watched per observation period; total of 860 minutes of observations). All observations were conducted between 0800 and 1630 hours on sunny days with minimal cloud cover and wind.

Analyses

Transplant experiment: All analyses were conducted in R (R Core Team 2013). We used *aster* life history models (Geyer et al. 2007; Shaw et al. 2008) in R (R Core Team 2013) to evaluate the effects of site, source population, and caging treatment on lifetime fitness, analyzing years 1 and 2 separately. *aster* models use a graphical approach that links sequential components of lifetime fitness, each modeled with its appropriate statistical distribution. Our *aster* model incorporated six components of lifetime fitness (*nodes* in the graphical model) for this experiment: germination, early survival (March), late survival (May), survival to flowering, fruit production (i.e., did the plant produce any seed-bearing fruits), and total seed set. The first five components were modeled as Bernoulli variables (0,1), and total seed set was modeled as a zero-truncated Poisson variable:

$$\mathbf{1} \rightarrow \underset{(0,1)\ Bernoulli}{\boldsymbol{germination}} \rightarrow \underset{(0,1)\ Bernoulli}{\boldsymbol{early\ survival}} \rightarrow \underset{(0,1)\ Bernoulli}{\boldsymbol{late\ survival}} \rightarrow$$
$$\underset{(0,1)\ Bernoulli}{\boldsymbol{survival\ to\ flowering}} \rightarrow \underset{(0,1)\ Bernoulli}{\boldsymbol{fruit\ production}} \rightarrow \underset{zero-truncated\ Poisson}{\boldsymbol{seeds\ produced}}$$

For year 1, we built an *aster* model with site, source population, caging treatment, and all interactions as predictors; response variables are those associated with each component of lifetime fitness. To estimate the effects of each predictor on lifetime fitness, each predictor was fit at the level of total seed set in the model (Shaw et al. 2008). We did not include block in these models to avoid overfitting at latter life history stages, when the total number of surviving individuals at a site was sometimes very low. We used likelihood ratio tests (LRTs) comparing submodels to fuller models to test each predictor of interest.

Because very few plants survived at two sites outside the range in year 1, we could not model lifetime fitness at all sites simultaneously. This is due to inherent limitations of maximum likelihood parameter estimation (Geyer 2009) that arise when all records in one category of a predictor have the same response at one node of the *aster* graph (e.g., every plant in Site A that is alive in March is dead in May). To circumvent this issue, we added one additional record, producing one seed, to each site / block / source population / caging treatment combination. This allowed us to use one model to estimate lifetime fitness at all sites. These "pseudorecords" made only miniscule differences to overall average seed production at each site (max difference between average fitness calculated from dataset with only observed records and dataset including pseudorecords = 0.02 seeds). For year 2, we built *aster* models as above but also included a planting year term to account for differences in seeds planted in 2015 and 2016. We did not need to include pseudorecords for year 2.

We tested pre-planned contrasts of lifetime fitness for caged and uncaged plants at each site by using LRTs to compare an *aster* model with all main effects, and site × source population and site × caging interactions, to a submodel that did not include the specific coefficient of interest. For example, to test whether lifetime fitness of caged and uncaged plants was significantly different at the Center site, we compared the large *aster* model to a model where caged and uncaged plants at the Center site were combined into one category, which removes the *Center* : *Caging* coefficient from the model. We adjusted test *P*-values with a Bonferroni correction. We also examined potential local adaptation within *xantiana*'s range. Local adaptation of *xantiana* populations would be evidenced by either 1) local populations having higher lifetime fitness than foreign populations at a site ("local vs. foreign" criterion), or 2) populations having highest lifetime fitness at their home site ("home vs. away" criterion). We tested "local vs. foreign" contrasts using the LRT approach as explained for caging above, and "home vs. away" contrasts in a similar manner, but subset datasets to model only one source population at a time.

Components of lifetime fitness: Because site, source population, and caging factors likely influence lifetime fitness through different components of lifetime fitness, we also

tested the influence of these predictors at each life history stage separately. We used logistic regressions to test the effects of site, source population, caging, site × caging, and site × source population on our Bernoulli life history components (germination through fruit production). Using the same model structure, we used linear regression for seed production, with a log transform of the response. For germination, we included a site × planting year interaction to account for seed age (planted in year 1 or year 2), but because there was no influence of seed planting year on the following stage (early survival), we dropped this term for stages after germination. Because there was no support in either year for a significant source population × caging term in the *aster* models of lifetime fitness, we did not include that interaction in our fitness component models. These separate fitness component analyses are "conditional" in the sense that, for each component after germination, we only used the subset of records that survived the previous life history stage (e.g., analysis of the probability of survival to May only included those plants that survived to March).

Predicting mean lifetime fitness: The fitness metric of most interest was mean seeds produced per planted seed. Because we planted two seeds per cell, and culled extra germinants, fitness predictions from our *aster* model would be slightly inflated relative to this metric. Thus, we obtained predicted values for lifetime fitness and their associated standard errors by taking the product of germination probabilities (see above) and unconditional parameter estimates from a full *aster* model that did not include a germination node. Standard errors for these products were calculated using the Delta method (Buehler 1957).

Pollen limitation: We analyzed pollen limitation (PL) in three ways. We first calculated a PL metric for each control / supplemental pair by dividing the difference in control and supplemental seed set (supplemental − control) by the larger of those two values (in some cases supplemented flowers will produce fewer seeds than control flowers for reasons beyond the control of the researcher). The resulting metric is thus the percent difference in seed set between supplemented and control flowers (e.g., PL = 0.1 means that control flower seed set was 90% of supplemented seed set). We tested whether PL differed within and outside the range using a linear model with region and site (nested within

region) as predictors, and PL (calculated from pairs of flowers) as the response. We deemed PL significant in a region if the 95% confidence interval for that region's PL estimate did not overlap zero. Second, we used paired, one-sided t-tests to ask whether supplemented flowers set more seed than control flowers within and beyond the range. Finally, we used logistic regression to test whether the probability of a flower pair exhibiting pollen limitation (i.e., having a positive PL metric) differed among regions, with site nested within region.

Pollinator visitation rates for each observation period were calculated as the number of visits divided by the number of flowers watched during that period. To test whether pollinator visitation differed inside and outside the range, we used a Mann-Whitney *U* test, as residuals from a linear regression model were highly non-normal and transformations did not appreciably improve their distribution.

Joint effect of biotic interactions*:* We estimated the joint effect of herbivory and pollen limitation on mean lifetime fitness outside the range in year 2 using a simulation (we did not perform pollen supplementations in year 1). We used estimates of pollen supplementation effects for each site (see *Pollen limitation*, above) to inflate seed counts of caged plants in that site by that pollen limitation estimate (for the Just Beyond site estimated PL was -0.02, so we did not simulate any change in seed set). Then, using this simulated dataset, we obtained mean lifetime fitness predictions, using the procedure described above. Comparison of lifetime fitness estimates for uncaged plants without simulated pollen supplementation and caged plants with simulated pollen supplementation estimates the joint effect of these biotic interactions on fitness outside the range. Data and code for all analyses are archived in the Dryad Digital Repository.

RESULTS

Precipitation

Year 1 was much drier than year 2 with ca. 40% - 170% more growing season (October through June) precipitation in year 2 (Fig. 2.2; Appendix 2 Table S1). Mean precipitation for sites outside the range was 39% that of sites inside the range in year 1 (164 and 416 mm, respectively) and 57% that of sites inside the range in year 2 (413 and 722 mm,

respectively). Compared to long term trends at each site, precipitation in year 1 was above average inside the range but below average outside the range, resulting in a steep gradient across the range margin. Whereas, precipitation in year 2 was considerably above average at all sites.

Patterns of lifetime fitness

Year 1: In year 1, mean predicted lifetime fitness for *xantiana* was 150 times higher inside the range than outside the range (12.0 vs. 0.1 seeds / per planted seed, respectively; Fig. 2.3; Table 2.1). However, this difference was disproportionately driven by extremely high fitness estimates at the Center site, where mean fitness estimates were 12 times higher than those at the next highest site, Edge (32.5 vs. 2.7, respectively). Relatively high fitness at Center was in large part driven by high fecundity (Fig. 2.3g; Table 2.2). Outside the range edge, mean fitness was low (< 0.3) at all sites.

Site, source population, and caging treatment were all significant predictors of lifetime fitness, and the effect of source population differed across sites (Table 2.1). The highest rates of herbivory were recorded at the Edge and Beyond sites (20% and 28% of uncaged plants alive at March census eaten, respectively), with low herbivory (< 6%) at the two most interior sites. No herbivory was recorded at the other two sites outside the range limit, likely because so few plants survived (< 6 uncaged plants at either site in May). There was support for a positive, but modest main effect of caging on lifetime fitness (LR = 7.3; $P = 0.007$), but tests of caging effects within individual sites uncovered significant differences between caging treatments at only the Edge site, where caged plants had mean lifetime fitness ca. three times higher than uncaged plants (4.0 vs. 1.4 seeds per planted seed, respectively; adjusted $P = 0.02$).

There was evidence for local adaptation (*local* > *foreign* fitness and *home* > *away* fitness) of the Center population in year 1. At the Center site, predicted lifetime fitness of the Center population was more than double that of the Edge population (46.6 vs 18.4 seeds per planted seed, respectively; LR = 119.6; $P < 0.0001$; Fig. 2.3a). Center genotypes also performed best at their home site, Center (all $P < 0.0001$). Overall, there was no significant difference in performance of Edge and Center populations at the Edge

site (LR = 2.4; P = 0.12). However, when protected from herbivory, mean fitness of the Edge population was about twice that of the Center population at the Edge site, though this contrast was only marginally significant (LR = 3.5; P = 0.06; Fig. 2.3a). Mean lifetime fitness of Center and Edge populations did not vary significantly outside the range limit.

In analyses of individual life history components, site was a strong predictor of performance at each stage (Table 2.2), though geographic patterns varied through *xantiana*'s life history (Fig. 2.3). Probability of germination decreased from the center to edge, then increased further east beyond the range margin (Fig. 2.3b). Late season survival was generally low outside the range, especially at Just Beyond and Far Beyond sites, likely due to water scarcity as the season progressed (Fig. 2.3d). Fruiting plants at Center produced far more seeds than did plants at other sites (Fig. 2.3g). Neither *source population* nor the *source population* × *site* interaction were identified as significant predictors in germination or early survival models (Table 2.2; Fig. 2.3), suggesting that maternal environmental effects did not influence early life stages (where they are most likely to be realized) in a way that would confound overall results.

Year 2: In year 2, mean predicted lifetime fitness (across treatments) was relatively high at all sites (1.3 – 4.1 seeds produced per planted seed). Site and caging treatment were significant predictors of lifetime fitness, but there was not support for a main effect of source population; however, there were significant interactions of site × source population, site × caging treatment, and site × caging × source population (Table 2.1).

Overall, herbivory rates were higher in year 2 (Appendix 2 Table S1). Twenty percent of uncaged plants were eaten at the Intermediate site, and approximately 37% were eaten at Just Beyond and Beyond sites. The Far Beyond site had 12% of plants eaten. At the Center and Edge sites, plants were subject to herbivory at rates of ca. 4%. Comparisons of caging treatments within individual sites showed that caging was associated with 3-7 fold increases in lifetime fitness at the four sites with high herbivory rates (all $P < 0.0001$; range of LR: 36.2 – 67.6; Fig. 2.4a). There was no difference in fitness between caging treatments at the Edge site, where herbivory was very low. At Center, caging treatment was associated with a modest reduction in fitness (LR = 12.43;

P = 0.002), though this should be interpreted with caution given the disproportionate influence of one plant in the uncaged treatment, which produced almost four times the seeds as the next most fit plant.

The effect of source population on lifetime fitness varied across sites (Table 2.1). The only significant differences in fitness among source populations were at the Center site, where the Intermediate population had higher predicted fitness than either Center or Edge populations (both $P < 0.0001$). There was no evidence of local adaptation for any source population at any site, regardless of caging treatment. Seed planting year was also highly significant due to higher germination rates of seeds planted in year 2 (Table 2.3).

In analyses of individual life history components, site, caging, source population, and their interactions were all significant predictors at some stage(s) (Fig. 2.4; Table 2.2). Germination generally increased going from the center to outside of the range (Fig. 2.4b). The effects of caging were mostly realized in the latter life history stages, especially late survival to seed production (Fig. 2.4; Table 2.3), which was when the majority of herbivory occurred in the field.

***Pollen limitation*:** Pollen limitation (PL) differed significantly within versus outside the range ($F_{1, 161} = 4.43$; $P = 0.04$) in year 2. There was evidence for significant PL outside the range (mean: 0.08; 95% CI: 0.001 — 0.15), but not inside the range (mean: -0.07; 95% CI: -0.18 — 0.03). Paired, one-sided t-tests gave no evidence for supplemented fruits setting more seed than control fruits inside the range ($t_{57} = 1.82$, $P = 0.96$), but indicated supplementation increased seed set outside the range ($t_{113} = -2.36$, $P = 0.01$). Logistic regression also showed that the probability of pollen limitation was 77% higher outside the range than inside the range (probability within: 0.32; probability beyond: 0.55; $P = 0.008$). Pollen limitation increased with distance from the range limit (Just Beyond, mean PL = -0.02; Beyond, PL = 0.07; Far Beyond, PL = 0.19).

Pollinator visitation rates differed strongly between regions ($W = 428$, $P < 0.0001$). Visitation was significantly higher inside the range (visits per flower per 10 minutes: mean = 0.80; median = 0.33) than outside the range (mean = 0.06; median = 0.00).

***Joint effects of biotic interactions outside the range limit*:** Amelioration of herbivory (*in situ*) and pollen limitation (*in silico* based on field estimates) together increased predicted mean lifetime fitness 3-7 fold outside the range edge (Just Beyond: 630% increase relative to uncaged plants with no simulated pollen supplementation; Beyond: 342%; Far Beyond: 251%). At all sites, this fitness increase was primarily due to prevention of herbivory, but pollen supplementation *in silico* further increased lifetime fitness at the Beyond and Far Beyond sites by 7 and 19 percent, respectively.

DISCUSSION

Evolutionary and ecological studies of species distributions often make assumptions about climatic factors primarily driving range limits, but there is no *a priori* reason to think biotic interactions are of any less import for determining geographic range boundaries. Given their demonstrated effects on local adaptation and population dynamics, the *potential* for biotic interactions to influence large scale distributions is increasingly discussed in the literature (e.g., Araújo and Luoto 2007; Van der Putten et al. 2010; HilleRisLambers et al. 2013; Wisz et a. 2013; Hargreaves et a. 2014; Godsoe et al. 2015; O'Brien et al. 2017; Bridle et al. 2019.). However, there is a paucity of studies that move beyond correlational approaches to examine the influence of species interactions on range limits (Louthan et al. 2015). In the present study, we found that biotic interactions contribute significantly to maladaptation beyond the range limit of *xantiana*, where abiotic conditions become increasingly stressful. There was also a strong temporal abiotic $\times$ biotic interaction — the effects of fatal herbivory were strongest when high precipitation led to relatively benign abiotic conditions beyond the range limit. Together these results illustrate how the interplay of abiotic and biotic factors across complex environmental gradients can limit species' geographic distributions.

Fitness variation in the absence of plant-animal interactions

Variability in precipitation drove both temporal and spatial variation in *xantiana* fitness. During year 1, sites within the range received near or above average precipitation, while sites outside the range received below average rainfall. Those sites outside the

range limit received less than half the precipitation than did sites inside the range, which led to relatively high *xantiana* fitness at all sites inside the range limit, and mean fitness near zero outside of it (Appendix 2 SI.5, Fig. S1). At Center, relatively strong performance at all life history stages led to high fitness, but the site was most differentiated from other within-range sites by high fecundity (seed set), which resulted from the large size of plants at this site (on average, fruiting plants at Center produced ca. 4 times the number of fruits as plants elsewhere within the range). Outside the range edge, low fitness mainly resulted from a combination of low germination rates, low late season survival, and low fecundity.

In year 2, all sites received above average growing season rainfall, which led to high mean lifetime fitness at all sites. In contrast to year 1, for uncaged plants, mean fitness was relatively equal within and beyond the range edge. Sites inside the range tended to have lower germination but higher late survival than sites outside the range. This relative parity of performance between regions was realized even though the two regions still received substantially different amounts of rainfall in year 2 (70% more rainfall within range). Supplementary analyses support the idea that lifetime fitness tended to decrease with increasing precipitation in year 2, when water availability was not likely limiting (Appendix 2 SI.5; Fig. S1). This pattern suggests that plants outside the range edge were able to better capitalize on the adequate water resources of year 2 than plants inside the range, potentially because of less severe competition with other forbs and grasses. For example, the site where fitness was highest in year 1, Center, had much lower mean fitness in year 2, largely due to a reduction in germination rates. Observations lead us to believe that this was likely due to vigorous growth of annual grasses at the site (i.e., plant - plant interactions). These grasses germinate early and grow quickly after the first winter rains, and in year 2 could have either prevented germination or caused early death of young *xantiana* germinants (which we could not observe and thus scored as a lack of germination).

For a species distributed across a continuous environmental gradient, it is generally thought that dispersal and subsequent local adaptation of peripheral populations to that gradient will enable range expansion (Mayr et al. 1963; Kirkpatrick and Barton

1997; Bridle and Vines 2007; Polechová 2018). We found evidence of strong local adaptation for the Center population in year 1, but not for the Edge population, and no indication that Edge populations fared better outside the range limit. Interestingly, there was a trend of the Edge population outperforming the Center population at the Edge site when protected from herbivory ($P = 0.06$). This suggests that the Edge population may be locally adapted to other aspects of the Edge site, such as lower precipitation, but not to increased fatal herbivory at the range edge. Adaptation to local conditions at the range edge, and the associated increase in population size, has important demographic (e.g., export of colonists) and genetic (e.g., increased variation, reduced influence of drift, potential "pre-adaptation") consequences for range expansion (Kawecki 2008). Geber and Eckhart (2005) also found no evidence of regional adaptation of edge *xantiana* populations; this lack of a signal of adaptation to the local environment at the range edge merits consideration. Theory predicts that lack of local adaptation in edge populations could arise from maladaptive gene flow from more abundant central populations (Haldane 1956; Antonovics 1976; Kirkpatrick and Barton 1997), or from the fact that smaller edge populations simply harbor less genetic variation upon which selection can act or are more prone to the effects of genetic drift (Whitlock 2004; Sexton et al. 2011). For *xantiana*, despite very high effective population sizes at the range edge, edge populations do exhibit modest reductions in private alleles (Moeller et al. 2011), though this may not be a good analog for quantitative genetic variation in ecologically-important traits (Reed and Frankham 2001). Moeller et al. (2011) also found some evidence of asymmetric gene flow from central to edge populations, which could potentially "swamp" locally adaptive alleles over long time scales. Furthermore, recent theory has highlighted how the demographic cost of such maladaptive gene flow can enable genetic drift to overpower selection in range edge populations, leading to the formation of a range limit (Polechová and Barton 2015; Polechová 2018). These hypotheses deserve further attention in *xantiana*.

In year 2, there was no evidence for local adaptation of any population. Though we certainly expect local adaptation of many populations (Antonovics 1987), we should not expect it to be temporally consistent if the environment is not (O'Brien et al. 2017;

Brady et al. 2019). Homing in on an optimal phenotype is made more difficult by temporal variation in selection (Milner et al. 1999; Kirkpatrick and Peischl 2013; Hao et al. 2015), and it is unlikely for a phenotype to arise anywhere that is optimally adapted to all possible conditions at that site across years. Rather, theory predicts that fluctuating environments will often select for "intermediate" phenotypes that perform best in the "average" environment (Sæther and Engen 2015).

Biotic interactions limit fitness beyond the range edge

One major goal of this study was to estimate the effect of an antagonistic biotic interaction, fatal mammal herbivory, on lifetime fitness in *xantiana* using experimental field manipulations within and outside the subspecies' range. Previous work has identified two lagomorph species, the desert cottontail (*Sylvilagus audubonii*) and the black-tailed jackrabbit (*Lepus californicus)*, as the primary herbivores causing mortality of *xantiana* inside and outside its range (Geber & Eckhart 2005; Benning et al. 2019). In the first year of our experiment, herbivory had little effect on mean lifetime fitness except at one site with high rates of herbivory (Edge), and geographic patterns in fitness largely reflected spatial variation in rainfall. In year 2, when experimental populations were partially "released" from abiotic limitations due to increased rainfall, protection from herbivores had large effects on mean fitness at all sites outside the range, and one site inside the range. For a *xantiana* population to persist outside its current range limit, low mean fitness in low precipitation years would have to be offset by high mean fitness in high precipitation years. As noted in Geber & Eckhart (2005), *xantiana* population persistence in hypervariable environments like the southern Sierra Nevada likely depends on both seed dormancy to temper the effects of "poor" years, and large inputs into a seed bank in years of favorable climate (e.g., Templeton and Levin 1979; Pake and Venable 1996). In this study, uncaged plants outside the range had the capacity to capitalize on adequate water resources in the high precipitation year 2, and despite herbivory, achieved mean fitness levels roughly on par with sites inside the range. However, protection from herbivory outside the range led to 3-7 fold increases in mean fitness of caged plants, resulting in fitness estimates even higher than those inside the range.

Demographically, this large fitness increase due to release from herbivore pressure would provide an additional buffer against years of poor abiotic conditions outside the range limit. For annual plants like *xantiana*, population mean lifetime fitness approximates population growth rate. It is interesting to note that at sites with high herbivory in year 2, only caged plants had mean lifetime fitness values whose 95% confidence intervals exceeded one (i.e., demographic replacement). In terms of adaptive evolution, any increase in effective population size would also make it more likely that colonizing populations of *xantiana* would adapt to the novel environment outside the current range edge, and decrease the influence of drift in colonizing populations (Kawecki 2008). Not only do herbivores have the potential to reduce *xantiana* population sizes, in this experiment they also preferentially ate larger plants (SI.4). Given that a large *xantiana* individual can produce thousands of seeds, herbivore preference for large plants could result in an outsized effect on mean population fitness.

The results of our pollen limitation experiment showed that while pollen limitation was both more likely and significant outside the range limit, the magnitude of pollen limitation was somewhat modest — supplemented flowers set, on average, 8% more seeds than unsupplemented controls outside the range limit. Estimates of PL increased with increasing distance beyond the range edge, with the strongest effects of supplementation (PL = 0.19) at the Far Beyond site. This likely reflects the parallel geographic trend of decreasing pollinator abundance and diversity that we found previously (Moeller 2006; Moeller et al. 2012). The results of this experiment suggest that pollen limitation is unlikely to be high enough to, on its own, prevent *xantiana* populations from establishing outside the subspecies' eastern range limit. It is important to note, however, that a colonizing population of *xantiana* would be significantly smaller than those in the current experiment, and past evidence indicates that reproduction can be strongly pollen limited in small populations (Moeller 2004; Moeller and Geber 2005).

Gradients in biotic interactions

Recent evolutionary models suggest that range limits can be mainly explained by the influence of genetic drift on leading-edge populations, and the fitness cost of

migration beyond the current range limit (Polechová and Barton 2015; Polechová 2018). The fitness cost of migration is determined by the steepness of the relevant environmental gradient, which determines the rate at which the optimal phenotype shifts. One of the most interesting patterns to emerge from these and another recent theoretical inquiry (Bridle et al. 2019) is how nonlinear environmental gradients seem to be essential for the collapse of local adaptation and the formation of an abrupt range edge. Both this transplant experiment and our previous work mapping spatial gradients in herbivory (Benning et al. 2019) demonstrate that the probability of fatal herbivory increases sharply near *xantiana*'s eastern range limit. Our previous work also provided evidence that this steep, nonlinear gradient in herbivory is tied to a steep cline in optimal phenology, with faster development enabling escape from herbivory and therefore favored outside the range (Benning et al. 2019). Other work has shown that the abundance of pollinators declines sharply along this same spatial gradient, with *Clarkia* specialist bees dropping out completely beyond the range limit (Moeller 2006), which may result in a similarly steep gradient in optimal values of floral traits like herkogamy (Moeller and Geber 2005; Moeller 2006). These steep gradients in optimal phenotype may be too great an adaptive hurdle for colonizing *xantiana* populations to overcome.

Abiotic × biotic interactions

In a recent paper, Louthan et al. (2015) reviewed the "Species Interactions - Abiotic Stress Hypothesis" (SIASH), which posits that biotic interactions will dominate in abiotically benign environments, while abiotic conditions will largely control population growth in abiotically stressful environments (sensu Darwin 1859; Dobzhansky 1950; MacArthur 1972). The results of the current experiments do not align with the SIASH, at least interpreted in a spatial context as it classically is — most measures of "stress" on population growth rate tend to vary collinearly for *xantiana*. As one moves from the center of the range to the eastern edge, rainfall decreases and becomes more variable, mammal herbivory increases, as does pollen limitation. However, our results do align with SIASH in a *temporal* context — biotic interactions were relatively more important, in terms of their effects on mean fitness, in year 2 when the abiotic conditions

outside the range edge were more benign. Thus, the negative effects of herbivory were realized most strongly in years that would be the most important for promoting long-term persistence outside the range limit, a finding consistent with a meta-analysis of the effects of invertebrate herbivory on plant population growth (Katz 2016).

Conclusion

The conclusion that "range limits are complex" perhaps should not surprise us, given the demonstrated power of natural selection to produce adaptations to novel environments. The results above highlight what Joseph Grinnell observed in 1917 — geographic range margins are multifaceted phenomena that will most often result from multiple, interacting factors. In addition, the relative importance of these factors can vary temporally, requiring multiple year studies to detect. Though correlative approaches can provide initial insights into the environmental variables associated with species' distributional limits, manipulative experiments are necessary for robust tests of specific hypotheses. For *xantiana*, transplant experiments have suggested that limited precipitation contributes to maladaptation beyond the range limit. As in many systems, this was simple to predict given that the range limit falls along an obvious abiotic gradient. Much less obvious was the spatial gradient in fatal herbivory that drove severe fitness losses beyond the range boundary, particularly in the abiotically favorable year when population growth rates could potentially be high. Generally, gradients in biotic interactions are easy to overlook given that many are difficult to measure without significant field efforts, and/or vary collinearly with abiotic gradients. However, these interactions may be pivotal in explaining the sharp geographic gradients in fitness that theory predicts are most likely to prevent range expansion (Polechová 2018; Bridle et al. 2019).

Chapter 3

Plant - soil microbe interactions across and beyond the geographic range of *Clarkia xantiana* ssp. *xantiana*

ABSTRACT

Interactions between plants and soil dwelling fungi and bacteria are ubiquitous, and these interactions have large effects on individual plant fitness. However, the degree to which spatial variation in soil microbial communities contributes to observed large scale patterns in plant population growth and species' distributional patterns remains largely untested. Using the California native plant, *Clarkia xantiana* ssp. *xatiana*, we paired greenhouse and field reciprocal transplants of plant source populations and soils to quantify plant local adaptation to soil biota and ask whether spatial variation in soil microbial communities could contribute to the plant's geographic range limit. Microbial communities from one site within *C. x. xantiana*'s range had positive effects on components of fitness in both the greenhouse and field experiment, and there was no evidence of local adaptation of plant populations to their local microbial communities. Pairing these experimental data with amplicon sequencing of microbial communities indicated the potential for enemy release outside *C. x. xantiana*'s range boundary, and that patchily distributed mutualists may influence geographic variation in fitness inside and outside the subspecies' distribution.

INTRODUCTION

Soil microbial communities are extraordinarily diverse and can exhibit high turnover rates at both small (e.g., Wolfe *et al.*, 2007) and large (Fierer & Jackson, 2006; Caporaso *et al.*, 2011; Tedersoo *et al.*, 2014) spatial scales. The effects of these microbes on plants has been a vigorous research area for decades, and there is much evidence that soil bacteria and fungi play integral roles in plant growth (e.g., Klironomos, 2003; Kennedy *et al.*, 2007; Lau & Lennon, 2012; van der Heijden *et al.*, 2016), phenology (e.g., Wagner *et al.*, 2014), and reproduction (e.g., Wolfe *et al.*, 2005) in a variety of habitats (Lugtenberg & Kamilova, 2009; Anderson *et al.*, 2010; Hayat *et al.*, 2010; reviewed in Augé, 2001). Unsurprisingly, the effects of microbes on individual plants scale up to influence community and ecosystem level processes (van der Heijden *et al.*, 1998; Klironomos, 2002; Van Der Heijden *et al.*, 2008; Bever *et al.*, 2015; Jasper Wubs *et al.*, 2016; Mommer *et al.*, 2018). Given the ubiquity of these interactions and their myriad effects, the potential for soil biotic communities to influence the dynamics of plant populations is high. In particular, spatial variation in microbial mutualist and pathogen communities could contribute to spatial patterns in plant fitness, local adaptation of populations, and the distributions of plant species (Thrall *et al.*, 2007; Van der Putten, 2012).

Within a plant species' range, geographic variation in the biotic environment may result in local adaptation of plants to their home soil microbial communities (Revillini *et al.*, 2016). Alternatively, plant populations may be maladapted to their local soils, for instance due to pathogen specialization on local plant genotypes. Thus far, evidence for plant - soil microbe local adaptation is mixed (e.g., Klironomos, 2003; Sherrard & Maherali, 2012; Rúa *et al.*, 2016; Revillini *et al.*, 2016; Manzanedo *et al.*, 2018), and it is unclear whether adaptation or maladaptation is the predominant pattern. The best evidence comes from studies focusing on mycorrhizal fungi, a group of symbionts shown to mediate local adaptation in several plant taxa (Stahl & Smith, 1984; Schultz *et al.*, 2001; Johnson *et al.*, 2010; Pickles *et al.*, 2015; reviewed in Rúa *et al.*, 2016). In testing for local adaptation of plants to soil microbes, researchers either isolate specific groups of microbes (e.g., mycorrhizal fungi, rhizobia, etc.) to use in experimental inocula, or take a

"whole community" approach. The benefit of experiments with whole soil communities is that they can capture the complex web of positive and negative plant - microbe interactions that occur in nature, which may be especially important if microbial effects are dependent on microbial community context (e.g., Hoeksema *et al.*, 2010). Experiments focusing on whole soil microbial communities have shown both adaptation and maladaptation of plant populations to their local whole soil communities (Smith *et al.*, 2012; Sherrard & Maherali, 2012; Lankau, 2013; Pickles *et al.*, 2015), or even aspects of both patterns simultaneously (e.g., Lankau & Keymer, 2018).

The environmental variables that structure adaptation *within* a species' range may or may not be those that contribute to the taxon's geographic range limit. Maladaptation to environments outside their distributional limit likely prevents range expansion in many species (Angert & Schemske, 2005; Geber & Eckhart, 2005; reviewed in Lee-Yaw *et al.*, 2016), though limited dispersal and/or landscape barriers also contribute to range boundaries (Svenning & Skov, 2007; Alexander *et al.*, 2018). By observation of individuals in range edge populations, or populations transplanted outside the natural range boundary, we can often directly observe that these peripheral environments are "stressful" relative to regions more central to the species' geographic range (Angert, 2006; Eckhart *et al.*, 2010; Louthan *et al.*, 2013). However, it is difficult to know which abiotic or biotic factors cause a range limit to occur where it does (Gaston, 2009). Theory supports the potential role of biotic interactions in setting these limits (Hochberg & Ives, 1999; Case & Taper, 2000; Gravel *et al.*, 2011), but compared to abiotic factors, few experiments test the idea that species interactions contribute to a geographic range boundary (but see Stanton-Geddes & Anderson, 2011; Afkhami *et al.*, 2014; Baer & Maron, 2018; reviewed in Louthan *et al.*, 2015).

Every plant that germinates in soil begins life in a microbial milieu. For plant populations in marginal habitats, soil microbial communities may be especially important in modulating environmental stress caused by other abiotic [e.g., nutrient limitation; (Johnson *et al.*, 2010)] or biotic [e.g., herbivory; (Garrido *et al.*, 2010)] factors. Low abundance of important mutualists at or beyond the range edge has the potential to influence the location of plant species' boundary (e.g., Nuñez *et al.*, 2009; Peay *et al.*,

2010; Stanton-Geddes & Anderson, 2011; Lankau & Keymer, 2016; Osborne *et al.*, 2018); this pattern could arise through two non-mutually exclusive mechanisms. First, soil mutualists could respond independently to environmental gradients across their host's range [e.g., arbuscular mycorrhizal fungi abundance decreasing with aridity (Yang *et al.*, 2011)]. Second, low abundance of compatible mutualists could be an emergent pattern arising from a lack of positive plant-soil feedbacks, where host population densities are too low at the range edge (and absent beyond it) to support abundant soil mutualist communities (Parker, 2001). Alternatively, novel pathogen taxa or genotypes at or beyond the range edge could depress peripheral population growth rates or prevent colonization outside the range, due to a lack of coevolutionary history between plant hosts and soil pathogens (Parker & Gilbert, 2004; Lankau & Keymer, 2018).

The potential role of microbes in contributing to the geographic range limits of native plant species has received startlingly little attention. In the only field experiment to date, Stanton-Geddes & Anderson (2011) found that for a legume transplanted outside its northern range edge, inoculation with rhizobial bacteria increased root colonization outside the range but not within; however, inoculation had similar effects on plant growth within and beyond the range. Lankau & Keymer (2016) documented that the richness of ectomycorrhizal fungi colonizing two tree species decreased with distance from the range center, suggesting that depauperate fungal communities may contribute to the range limit of these taxa. Osborne *et al.* (2018) presented convincing evidence that divergent adaptation to arbuscular mycorrhizal fungal communities between two soil types contributes to niche divergence and coexistence in the two species of *Howea* palms on Lord Howe Island. Interestingly, the role that soil microbes play in regulating *exotic* plant invasions is perhaps more widely discussed than their effects on native species' geographic ranges. Empirical examples include lack of ectomycorrhizal fungi limiting invasion of exotic pines in Argentina (Nuñez *et al.*, 2009), and release from virulent oomycetes aiding the invasion of an exotic *Prunus* in Europe (Reinhart *et al.*, 2010). These examples illustrate the potential of plant - soil microbe interactions to influence plant distributional limits, and highlight the need for more research, especially

experiments that can test the influence of plant-microbe interactions on plant fitness in the field.

Most experimental work with plant - soil microbe dynamics takes place in the laboratory or greenhouse (but see Johnson *et al.*, 2001; Parker *et al.*, 2006; Stanton-Geddes & Anderson, 2011). Though there are often calls for more realistic manipulative field experiments (e.g., Dawson & Schrama, 2016) given that plant phenotype can vary strongly between field and greenhouse (e.g., Poorter *et al.*, 2016), and that many plant-microbe interactions are context dependent (e.g., Hoeksema *et al.*, 2010), few have been executed. Thus, even though controlled conditions can aid in, for example, isolating the effects of pairwise interactions between plant and microbial taxa (e.g., Klironomos, 2003), our understanding of the effects of microbes in natural plant populations is limited by a lack of field studies.

Using a California annual plant, *Clarkia xantiana* ssp. *xantiana* (hereafter, *xantiana*), we reciprocally transplanted plant populations and soil microbial communities in the greenhouse and field to test for plant - soil microbe local adaptation and the potential of soil microbes to contribute to the plant's geographic range limit. *Xantiana* is endemic to the southern Sierra Nevada foothills, a region with extraordinary plant and animal diversity and small scale heterogeneity in climate and soils. Transplant experiments have demonstrated that *xantiana*'s geographic range is most likely limited by adaptation, not dispersal (Geber & Eckhart, 2005), and high environmental heterogeneity has driven population divergence in important phenotypic traits such as phenology and size (Gould *et al.*, 2014). One of the most obvious environmental drivers in this system is precipitation, with a spatial trend of increasing aridity toward *xantiana*'s eastern range edge, and a temporal trend of decreasing water availability as the growing season progresses (Eckhart *et al.*, 2010, 2011; Benning *et al.*, 2019). Because soil microbial communities can directly influence plant water relations (e.g., Augé, 2001; Lau & Lennon, 2012) and/or affect traits related to drought avoidance (e.g., phenology: Wagner et al. 2014), we were especially interested in how soil microbes may modulate local and range adaptation across and beyond the precipitation gradient that underlies *xantiana*'s distribution.

In the greenhouse, we grew multiple *xantiana* populations with soil microbial communities sourced from those same populations, and sites outside *xantiana*'s range limit, in a full factorial design, and used amplicon sequencing to characterize microbial communities in the different field soils. In the field, we transplanted multiple *xantiana* populations into six sites within and beyond its range, where plants grew with one of three soil microbial inocula sourced from inside the range, or a local control. We used these experiments to answer three main questions: 1) how does geographic variation in microbial communities affect *xantiana* growth and phenology? 2) Do *xantiana* populations show evidence of local adaptation or maladaptation to their soil microbial communities, and/or maladaptation to microbial communities outside their geographic range limit? 3) Are the effects of microbial communities on plant growth and fitness similar in the greenhouse and field?

METHODS

Study system

Clarkia xantiana ssp. *xantiana* A. Gray (hereafter, *xantiana*) is a winter annual native to the Southern Sierra Nevada foothills of California, USA (Eckhart and Geber 1999). *Xantiana* is distributed across an aridity gradient (with precipitation lower and more variable toward and outside its eastern range edge) that contributes to reduced performance at the range edge and beyond (Eckhart et al. 2010, 2011). The region receives the majority of its precipitation in winter, with little to no precipitation falling after April (Eckhart *et al.* 2011). After germinating in the relatively wet winter (November - December), *xantiana* grows throughout the spring and sets seed in late June. Western and central *xantiana* populations are found primarily on steep slopes in relatively mesic oak woodlands of the Kern River Canyon, and eastern edge populations are found in drier pine woodlands (Eckhart *et al.* 2011; Gould *et al.* 2014). Most populations, including all used in this study, are found on sandy soil derived from igneous rock (Eckhart *et al.*, 2010). The eastern range edge is stark and extensive searching over the past 20+ years has uncovered no *xantiana* populations beyond this limit.

In contrast to most study systems, the influence of biotic interactions on *xantiana*'s distribution has received a relatively large amount of attention in several within and beyond-range experiments. These studies have shown that mutualistic interactions with pollinators are weaker at and beyond the range limit, resulting in greater pollen limitation of reproduction for this primarily outcrossing taxon (Moeller *et al.*, 2012; Anderson *et al.*, 2015; Benning *et al.*, 2019). By contrast, antagonistic interactions with mammalian herbivores are stronger at and beyond the range limit (Benning *et al.*, 2019). Field observations have confirmed that *xantiana* is colonized by arbuscular mycorrhizal fungi (Benning; pers. obs.), but the effects of geographic variation in soil microbial communities on *xantiana* fitness remain untested.

Greenhouse experiment

We used a greenhouse experiment to test the influence of geographic variation in soil microbial communities on *xantiana* growth and phenology, and ask whether effects varied among plant populations. As opposed to the field experiment (see below), the greenhouse experiment allowed us to fully control the soil environment in which plants were grown. We grew seeds from three source populations with five soil microbial inocula (plus a control, described below) in a full factorial design. Seeds were collected haphazardly from 16 maternal plants from each of three populations in the field in June 2016. The three populations were at *xantiana*'s range center (Center), between the center and range edge (Intermediate), and near the eastern range edge (Edge) (Fig. 3.1). In November 2016, soil was collected from the three sites above, and two sites outside the range (Just Beyond and Beyond sites, 5 and 14 km outside the eastern range edge respectively), to use as experimental inoculum. Soil was collected from the top ca. 30 cm at multiple (> 5) locations to sample from the main extent of natural *xantiana* populations within each of the five sites, and kept at 4° C until shipping to the University of Minnesota, where it was refrigerated upon arrival until the experiment began in December 2016. Each site's soil was homogenized before use.

In order to maintain similar nutrient levels across inocula, each experimental inoculum consisted of 20% "live" focal inoculum and 80% of an even mix of the other

four inocula sources, which were autoclaved. We autoclaved field soil for 1 hr at 121° C, allowed it to rest overnight, and autoclaved for another 1 hr at 121° C. The control inoculum consisted of all five (autoclaved) inoculum sources. Thus, there were six total inoculum treatments, each comprising soil from all inoculum sources, but with different "live" inocula. All experimental inocula mixtures were homogenized before transferring to pots.

We grew plants in 983 cm^3 D60 Deepots (Stuewe & Sons, Oregon, USA). We crumpled newspaper to cover drainage holes at the bottom of the pot, and then steamed all pots (with newspaper) for 2 hrs at 80° C before filling with soil. The soil mix for each pot comprised 400 cm^3 of the mixed inoculum described above, with 270 cm^3 of sand (twice steamed at 80° C for 2 hrs). The inoculum and sand were poured into pots simultaneously so that the inoculum/sand mixture was distributed throughout the length of the pot. In December 2016, two seeds were sown on top of the soil in each pot, completely randomizing the location of all treatment combinations within the greenhouse. We culled one germinant if both seeds germinated. Not all pots had germinants. In 43 cases, we transplanted the second germinant from a pot with two germinants to an empty pot (within the same population / inoculum treatment combination) to increase replication. Final sample sizes were 18 - 30 replicates for each combination of population and inoculum combination (mean = 25; N = 452).

To simulate the limited soil moisture conditions of *xantiana* in the field, each plant received 30 mL of reverse osmosis water per week. We measured the date of first flower, root biomass, and the total number of leaf nodes (and thus, number of leaves produced) at flowering (a proxy for aboveground biomass, as *xantiana* begins to senesece leaves early in its life cycle). Leaf number is well correlated with seed production in the field (r = 0.67; Benning, unpubl. data).

Statistical Analyses

All code for bioinformatics and statistical analyses will be stored in the Dryad Digital Repository. We analyzed the effects of plant population, inoculum, and their interaction on phenotypic traits (days to first flower, root biomass, and node number) using linear fixed effect models. Days to first flower was calculated as the number of

days that elapsed between the emergence of the first true leaf and the opening of the first flower. We also included a *bench* term to account for differences among the four growing benches in the greenhouse. Terms identified as significant with a Type II ANOVA were further explored with Tukey HSD tests to test for differences between treatment levels.

We used pre-planned contrasts based on estimated marginal means from our linear model of node number (a fitness proxy) to explicitly test for population local adaptation to soil microbial communities within *xantiana*'s range. To test for local adaptation under the *local vs. foreign* paradigm, we computed pairwise contrasts among populations within each of the three within-range inoculum treatments. Local adaptation under this paradigm would be indicated by each population outperforming the others when grown with their local microbial inoculum (e.g., Intermediate plants outperform Center and Edge plants when all are grown with Intermediate inoculum, etc.). To test for local adaptation under the *home vs. away* paradigm, we computed pairwise contrasts among the three within-range inoculum treatments within each plant population. Here, local adaptation would be indicated by populations obtaining their highest fitness values when grown with their local microbial inoculum. To test whether there was an overall effect of microbial communities from inside (Center, Intermediate, and Edge sites) vs. outside (Just Beyond and Beyond sites) the geographic range on plant performance, we built a linear model of node number with plant population, inoculum region (inside vs. outside), and their interaction as predictors, again including a bench term to account for spatial variation in the greenhouse. This analysis did not include plants in the "control" inoculum treatment. We used Type II ANOVA to test the significance of each predictor in the model.

Soil DNA extraction

We extracted DNA from the soils used as inoculum in the experiment using the Qiagen DNeasy PowerSoil kit. We extracted two genomic DNA samples from each of the five homogenized inocula, and followed manufacturer protocols with a few modifications. First, we used a concentration step to extract DNA from a larger volume of inoculum soil than the 250 mg allowed by the kit. We again homogenized inoculum soil samples and added 15 g soil to a 50 mL tube. We then added 40 mL PBS-T, vortexed tubes for 15

seconds each, and then placed the tubes on an orbital shaker for 10 minutes at 150 rpm. The sample was then vortexed for 10 sec and then 11 mL was immediately transferred into a 13 mL tube. These subsamples were centrifuged at 1400 rcf for 15 minutes, and the supernatant discarded. We added 2 mL UltraPure water and vortexed before adding 500 uL of this concentrated sample to the kit extraction tubes. Samples were bead beat in PowerBead tubes with lysis buffer using a TissueLyser at 30 hz, for four two-minute sessions, allowing samples to rest for 60 sec between sessions, before following the remainder of the kit protocol. We extracted from both a negative control, and a positive fungal control (from Palmer *et al.*, 2018) processed with the kit extraction protocol to aid in downstream data cleaning (Nguyen *et al.*, 2015).

Amplification and Illumina sequencing

We used high throughput amplicon sequencing to characterize bacterial and fungal communities within the five inoculum sources. Sample DNA extractions were sent to the University of Minnesota Genomics Center for library prep and sequencing. Library prep was completed using the UMGC-developed dual indexing protocol (Gohl *et al.*, 2016), which has been shown to provide more quantitatively accurate and qualitatively complete measurements of microbial diversity in amplicon-based sequencing. For bacteria, we sequenced the V4 hypervariable region of the 16S rRNA gene, using the 515-F / 806-R primer pair. For fungi, we sequenced the ITS1 region of the rRNA gene using the ITS1-F / ITS2 primer pair. Primers included Illumina adapters and barcodes to enable multiplexing of samples. PCR products were pooled together in equimolar concentrations and sequenced on an Illumina MiSeq running 2 x 300 bp chemistry.

Microbial Bioinformatics

Reads were demultiplexed and adapters and primers were removed. Downstream processing was completed using the dada2 pipeline (Callahan *et al.*, 2016). Reads were filtered and trimmed using the filterAndTrim function in dada2 (V4 trimmed at 200bp forward, 160 bp reverse, maxEE = 2; ITS1 minimum length 50 bp; maxEE = 4), and exact amplicon sequence variants (ASVs) inferred using the dada2 sample inference algorithm, which models and corrects Illumina-sequenced amplicon errors (Callahan *et al.*, 2016). ASVs offer finer resolution than clustered sequences such operational

taxonomic units (OTUs) and are increasingly recommended for their improved reusability, reproducibility, and comprehensiveness (Callahan *et al.*, 2017; Knight *et al.*, 2018). Paired reads were merged, chimeras removed, and taxonomy assigned using the naive Bayesian classifier method (Wang *et al.*, 2007), which assigns bacterial and fungal taxonomy using the Silva (Glöckner *et al.*, 2017) and UNITE (Nilsson *et al.*, 2019) reference databases, respectively.

We used the phyloseq, vegan, and DESEQ2 packages for further cleaning of reads and analysis of microbial community composition. After joining 16S and ITS sequences into a single ASV table, we removed any ASVs with fewer than 10 total reads across all samples, those with no "Kingdom" taxonomic assignment, and those assigned to Domain Archaea. In the fungal mock community sample, all 12 expected fungal mock sequences were recovered with high read abundances, and none of these mock sequences were found in any other samples. There were three additional fungal ASVs found at low abundance in the mock community sample which were present at low abundance in other samples, and 12 bacterial ASVs. The abundances of any non-mock ASVs found in the fungal mock community were subtracted from each sample, as well as abundances of all ASVs found in the negative control (3 fungal ASVs; 419 bacterial ASVs; median ASV read abundance = 2; Nguyen *et al.*, 2015).

All samples were rarefied to the minimum sampling depth of 41,784 reads without replacement. We calculated ASV richness and diversity (Shannon's H index) for each inoculum source, pooling the two samples for each source. We calculated both Bray-Curtis distance and Jaccard similarity matrices for samples (calculated for the full community, as well as fungal and bacterial communities separately), and visualized community distance using principal coordinates analysis (PCoA) in phyloseq. We used PERMANOVA (*adonis* in vegan) to test for differences in community composition between within- and beyond-range sites. Based on the results from the field and greenhouse experiments, we were interested in identifying potentially important ASVs from the Intermediate site, and used the DESeq2 package to test for differentially abundant ASVs between the Intermediate inoculum and the other four inocula. We filtered ASVs using a False Discovery Rate of $\alpha = 0.01$ to correct for multiple tests.

Field transplant experiment

We paired the greenhouse experiment with a field experiment to estimate the effects of soil microbial communities, geography, and source population on *xantiana* lifetime fitness. We planted seeds from the same three *xantiana* populations as above into six sites spanning from the center to 22 km outside the natural distribution of *xantiana*. The six sites were at *xantiana*'s range center (Center), between the center and range edge (Intermediate), eastern range edge (Edge), 5 km outside the eastern range edge (Just Beyond), 14 km outside that edge (Beyond), and 22 km outside that edge (Far Beyond) (Fig. 3.1). The three sites within the range contain natural *xantiana* populations, and the three sites outside contain its sister subspecies, *C. x. parviflora*. The experiment was conducted across two growing seasons (2015-2016 and 2016-2017; hereafter, year 1 and year 2).

Xantiana seeds were sourced from the Center and Edge sites in year 1, and Center, Edge, and Intermediate sites in year 2. In year 1, seeds used for planting were all collected from the field (30 to 70 maternal families per population). Due to drought and thus low site productivity in 2016, we generated seed for year 2 planting in the greenhouse (26 to 30 maternal families per population). Seeds were bulked before planting (i.e., we did not keep track of maternal families).

At each of the six sites, we installed 120 plastic grids arranged into six blocks (20 grids per block) set into the natural vegetation matrix (N = 720 grids total). Grids were cut from white plastic diffusion screens (used for fluorescent light fixtures) and set onto the soil surface after scraping away the top ca. 1 cm of soil underneath to avoid contamination from the local *C. xantiana* seed bank. Grids comprised a 7 x 7 matrix of 3 cm x 3 cm cells (with ca. 2 cm high walls); only the inner 36 cells were planted to avoid potential edge effects. These grids allowed us to follow plants from individual seeds while maintaining a natural growing environment for the experimental plants.

The source populations were randomly assigned to cells within grids using three randomized planting schemes (5 cells per source population per grid in year 1, and 4 cells per source population per grid in year 2); each grid was randomly assigned to a scheme. Two seeds were planted per cell in October of each year (in year 2, all seeds were planted

into empty cells). Thus, in year 2, the experiment included newly planted seeds as well as any seeds that were planted in year 1 and did not germinate. We account for these different seed cohorts in our analyses.

Because completely controlling plants' soil environment was infeasible in the field, our inoculum treatment was an addition treatment, where we added various soil inocula to the planting grids. Of course, the resident soil microbial community remained in place, so this treatment could only *add* potential symbionts to the experimental plants' growing environment. Each grid was assigned one of 4 inoculum treatments — soil from Center, Intermediate, Edge, or Control. The non-control soils were collected from their respective sites in October 2015 just before seed planting. After removing the top 5 cm to prevent seed contamination, soil was collected from the top 30 cm at multiple points within the natural *xantiana* populations. These soil samples were homogenized before applying to grids. We applied ca. 750 mL of soil inoculum to each grid. For control grids, we collected soil from beside each block in the same manner as we did for inoculum treatments, and applied 750 mL to grids. We followed all treatments with a thin top layer of control soil to equalize soil depth within grids . In each block of 20 grids, Control inoculum was applied to eight grids, and each of the other three inocula was applied to four grids.

The experiment included a caging treatment to test the effects of fatal mammal herbivory on lifetime fitness; these results are reported elsewhere (Benning & Moeller, in press). For all analyses below, we excluded all plants that were eaten. Thus, lifetime fitness estimates should be interpreted as reflecting the influence of site, population source, and inoculum treatment in the absence of mammal herbivory.

We visited sites in February and March to score germination and early season survival and growth, respectively, and monitored late season survival and growth and total seed set May through June in each year. We estimated seed set in each collected fruit using a linear model that predicted fruit seed set as a function of individual fruit weight. A plant's lifetime fitness is equal to the sum total seeds contained in all of its fruits.

Statistical Analyses

Lifetime fitness analyses All analyses were conducted in R (R Core Team 2013). We used *aster* life history models (Geyer *et al.*, 2007; Shaw *et al.*, 2008) in R (R Core Team, 2013) to evaluate the effects of inoculum treatment on *xantiana* lifetime fitness, analyzing years 1 and 2 separately. *Aster* models use a graphical approach that links sequential components of lifetime fitness, each modeled with its appropriate statistical distribution. Our *aster* model incorporated four components of lifetime fitness (*nodes* in the graphical model) for this experiment: germination, early survival (March), fruit production (i.e., did the plant produce any seed-bearing fruits), and total seed set. The first 3 components were modeled as Bernoulli variables (0,1), and total seed set was modeled as a zero-truncated negative binomial variable:

$$\mathbf{1} \rightarrow \underset{(0,1)\ Bernoulli}{\boldsymbol{germination}} \rightarrow \underset{(0,1)\ Bernoulli}{\boldsymbol{early\ survival}} \rightarrow$$

$$\underset{(0,1)\ Bernoulli}{\boldsymbol{fruit\ production}} \rightarrow \underset{zero-truncated\ negative\ binomial}{\boldsymbol{seeds\ produced}}$$

For each year of the experiment, we built an *aster* model with site, population, inoculum treatment, and all interactions as predictors; response variables are those associated with each component of lifetime fitness. The aster model for year 2 also included a planting year term to account for differences between cohorts planted in 2015 and 2016. To estimate the effects of each predictor on lifetime fitness, each predictor was fit at the level of total seed set in the model (Shaw et al. 2008). We did not include block in these models to avoid overfitting at latter life history stages, when the total number of surviving individuals at a site was sometimes very low. We used likelihood ratio tests (LRTs) comparing submodels to fuller models to test each term of interest. When terms involving *inoculum* were identified as significant via LRT, we explicitly tested relevant contrasts using LRTs of reduced *aster* models. E.g., if *site* × *inoculum* was a significant predictor in the full *aster* model, we tested for inoculum effects at each site by subsetting datasets by site, testing for a significant *inoculum* effect via LRT, and following significant tests with appropriate pairwise contrasts between *inoculum* types.

Because very few plants survived outside the range in year 1, and there were few germinants at the Center site in year 2, we could not model lifetime fitness at all sites

simultaneously in either year. This is due to inherent limitations of maximum likelihood parameter estimation (Geyer, 2009) that arise when all records in one category of a predictor have the same response at one node of the *aster* graph (e.g., every plant in Site A that is alive in March produces no fruits). To circumvent this issue, we added one additional record, producing one seed, to each site / population / inoculum treatment combination in each year. This allowed us to use one model to estimate lifetime fitness at all sites in each year. These "pseudorecords" made only miniscule differences to overall average seed production at each site (max difference between average fitness calculated from dataset with only observed records and dataset including pseudorecords = 0.007 seeds).

A significant *site* × *population* × *inoculum* term in these *aster* models would indicate that the effects of inoculum on lifetime fitness of source populations differed among sites. This second order interaction would be of particular interest as it could signify source population local adaptation or maladaptation to soil inocula, an interaction that would be expected to differ across sites (i.e., the effect of local inocula for a population would be expected to differ between the population's local site, and a foreign site) and result in a significant three way interaction for lifetime fitness. If *aster* analyses indicated a significant three way interaction, we focused on two main questions: first, within the range, how does addition of soil microbial inocula from a *xantiana* population's home site influence fitness when growing in novel environments? Thus, at each site within the range, for the two foreign populations, we asked whether lifetime fitness differed between plants grown with control inoculum and those grown with their home site inoculum. If addition of their "home" inocula consistently improved fitness of populations when planted into foreign sites, relative to plants grown with control inocula, this suggests adaptation of populations to their local soil mutualists. If addition of "home" inocula consistently depresses fitness in foreign sites, this suggests maladaptation of populations to their local soil pathogens. (Of course, soil communities will contain symbionts with both positive and negative effects, so these results capture the "net" effect of all microbes contained in an inoculum.) Second, we asked whether the addition of any of the three soil inocula from within *xantiana*'s range improved lifetime fitness of plants

when planted outside the range limit. Thus, at each site beyond the range (Just Beyond, Beyond, and Far Beyond), for each population, we asked whether lifetime fitness differed between plants grown with control inoculum and those grown with each of the three within range inocula. Within the two families of pairwise contrasts (questions 1: 6 contrasts; question 2: 27 contrasts), we adjusted test *P*-values with a sequential Bonferroni (Holm) correction.

Individual life history components Because effects of soil microbial communities may manifest at different stages of a plant's life, we also tested the influence of inoculum treatment at each life history stage separately. We used logistic regressions to test the effects of site, population, inoculum, and all interactions, on our Bernoulli life history components (germination through fruit production). Using the same model structure, we used negative binomial regression to model seed production. These separate fitness component analyses are "conditional" in the sense that, for each component after germination, we used only the subset of records that survived the previous life history stage (e.g., analysis of the probability of fruiting only included those plants that survived to March). For germination, we included a *site* × *planting year* interaction in analyses of year 2 to account for seed age (planted in year 1 or year 2), but because there was no influence of seed planting year on the following stage (early survival), we dropped this term for stages after germination. If Type II ANOVA indicated a significant main effect of inoculum or a significant interaction of inoculum with other terms, differences between treatment levels were tested with Tukey HSD tests.

Predicting mean lifetime fitness The fitness metric of most interest was mean seeds produced per planted seed. Because we planted multiple seeds per cell, and culled extra germinants, fitness predictions from our *aster* model that includes germination as a simple bernoulli variable (the cell either contained germinant(s) or not) would be inflated relative to this metric. Thus, we obtained predicted values for lifetime fitness and their associated standard errors by taking the product of germination probabilities (estimated from the logistic regression for germination described above, which incorporated information on multiple seeds per cell) and unconditional parameter estimates from a full

aster model that did not include a germination node. Standard errors for these products were calculated using the Delta method (Buehler, 1957).

RESULTS

Greenhouse experiment

Traits Flowering phenology differed strongly among populations, with Edge populations flowering earlier (ca. 10 days) than Center or Intermediate populations (Table 3.1; Appendix 3 Fig. S1). The effect of inoculum source on phenology differed significantly among populations (*population* × *inoculum*, $P < 0.05$), though the only significant pairwise inoculum difference was between Edge plants grown with control inocula and Intermediate inocula, where plants grown with control inocula flowered on average 8 days earlier than those grown with Intermediate inocula.

Above and belowground growth differed strongly among populations and inocula, and the magnitude of inoculum effects were roughly equal to those of plant source population (Table 3.1; Figs. 3.2; Appendix 3 Figs. S2, S3). Center plants were largest (produced the most nodes and largest root biomass), and Edge plants were smallest (ca. 20% smaller than Center); Intermediate plants were intermediate between these two (Appendix 3 Figs. S2, S3). Plants grown with control inoculum were, on average, smaller above- and belowground than those grown with any "live" inocula, and plant size in control inoculum was highly variable. For within-range inocula, in general, plants grown with Intermediate inoculum produced more nodes and root mass than those grown with Center and Edge inocula (Fig. 3.2). For example, plants grown with Intermediate inoculum produced 14 percent more nodes than plants grown with Center or Edge inoculum, and 15 and 23 percent more root biomass, respectively, though after adjustment for multiple tests these contrasts were only significant for node number. Plants grown with inoculum from outside the range (Just Beyond and Beyond) tended to be larger than those grown with Center or Edge inocula, but on par with those grown with Intermediate inocula.

Local and Range Adaptation There was no evidence of local adaptation to soil microbial communities among *xantiana* populations (*population* × *inoculum* $P > 0.05$;

Table 3.1), which would be demonstrated by populations achieving their highest fitness when grown with their home microbial inocula (home vs. away local adaptation), or having higher fitness values than other populations when all are grown with the former's home microbial inocula (local vs. foreign local adaptation) (Appendix 3 Fig. S3). Additionally, there was no evidence that performance differed when comparing plants grown with within vs. beyond range inoculum (i.e., range adaptation; *inoculum region P* = 0.16; *population* × *inoculum region P* = 0.78; Fig. 3.2, Appendix 3 Fig. S3).

Field experiment

Years 1 and 2 differed markedly in precipitation (Appendix 3 Fig. S4), which was associated with large temporal variation in fitness. In year 1, precipitation was near or above average inside the range, and below average outside, resulting in low mean fitness outside the range edge. In year 2, precipitation was high across and beyond the range, which led to similar overall mean fitness within and outside the range. Fitness beyond the range edge was strongly limited by high rates of mammal herbivory in year 2. Results pertaining to herbivory, site, and population source effects are reported in Benning & Moeller (2019) and only discussed below when they are relevant to the interpretation of inoculum effects.

Year 1 The effects of inoculum source on lifetime fitness differed among sites in year 1 (significant *inoculum* × *site* term; Table 3.2) and were driven by inoculum effects at the Center and Edge sites (Fig. 3.3a). At the Center site, plants growing with inoculum from foreign sites tended to have higher lifetime fitness than those growing with local inocula, but these differences were not statistically significant ($P > 0.05$). The effect of inoculum source was significant at the Edge site, where plants grown with Center inoculum had the highest lifetime fitness ($P = 0.02$). Inoculum treatment did not have a significant effect on any of the four components of lifetime fitness in conditional analyses (Table 3.3).

Year 2 There was no main effect of inoculum treatment on lifetime fitness in year 2, but there was a significant second order interaction of *inoculum* with *site* and *population* (Table 3.2; Fig. 3.3b). However, there was no indication that addition of a

population's home inoculum consistently increased or decreased fitness relative to controls at sites within the range (Fig. 3.3b; Appendix 3 Table S1). Outside the range edge, there was no indication that source populations had consistent responses to inocula sourced from inside the range (Fig. 3.3b; Appendix 3 Table S1).

In conditional analyses, there was a significant effect of inoculum treatment on a plant's probability of producing fruit (given early survival) (Table 3.3). The inoculum source that resulted in the highest growth in the greenhouse, Intermediate, also resulted in the highest probability of fruit production in the field (Fig. 3.4). Plants grown with Intermediate inoculum were ca. 50% more likely to produce fruits than those grown with Edge inoculum, and ca. 25% more likely to produce fruits than those grown with Center or control inoculum, though only the former contrast was statistically significant when tested with Tukey's HSD test. This effect was especially pronounced at the Edge, Just Beyond, and Beyond sites (Fig 3.4). There was also a significant three-way interaction of site, population source, and inoculum treatment effects on seed set (Table 3.3), but there was no indication that this reflected local adaptation (Appendix 3 Fig. S5), and no pairwise contrasts between inoculum sources for source populations within sites were significant after correction for multiple tests.

Characterization of soil microbial communities

After filtering, inoculum samples from the greenhouse experiment had an average sample read abundance of 75,170 reads, and ASV accumulation curves were all saturated (Appendix 3 Fig. S6). After rarefaction to the sample minimum at 41,784 reads per sample, a total of 5,171 ASVs were recovered across all samples (4,198 bacterial, 973 fungal).

Overall, ASV richness and diversity was higher for within-range sites relative to beyond-range sites (average ASV richness within-range: 3,283; beyond-range: 2921; Table 3.4), though differences in diversity metrics were modest (within-range Shannon's *H*: 7.1; beyond-range: 6.7). Bacterial communities followed the same patterns as the overall community, but the fungal community from the Intermediate site was relatively less rich than the other two within-range sites (Center and Edge), with values more in line

with beyond-range sites. All sites had unique ASVs (range: 264 - 359 per site), with no apparent geographic pattern in number of unique taxa at each site. However, as a group, within-range sites had more than twice the number of unique taxa than beyond range sites — there were 260 ASVs unique to within-range sites (i.e., found within all within-range sites but no beyond-range sites), and 95 ASVs unique to beyond-range sites.

Ordinations of community composition using Bray-Curtis distances showed clear separation of within- and beyond-range sites along the first PCoA axis (which explained ca. 40% of compositional variation) (Fig. 3.5, Appendix 3 Fig. S7). PERMANOVA indicated that within- and beyond-range sites differed in composition at the level of the full microbial community, as well as for fungal and bacterial communities separately (all $P < 0.01$). Ordinations based on the Jaccard index produced similar groupings of within- and beyond-range sites (Fig. 3.5, Appendix 3 Fig. S7), indicating that patterns were not driven solely by relative abundance of taxa, but also by the presence/absence of individual taxa.

At higher taxonomic levels within the bacterial community, there were no obvious differences among sites in relative abundance of phyla (Appendix 3 Fig. S8). Within the fungal community, within-range sites tended to have more Chytridiomycota, and the Intermediate and Edge sites had the highest relative proportion of Glomeromycota (arbuscular mycorrhizal fungi), with Glomeromycota accounting for ca. 2% of all reads at the Intermediate site, 1% of all reads at the Edge site, and less than half a percent at each of the other three sites. Based on results from the greenhouse and field experiments, we were especially interested in whether there were ASVs for which the Intermediate site was significantly enriched. Differential abundance analyses indicated that 136 ASVs were significantly either over or under abundant in the Intermediate inoculum relative to at least one of the other inocula (Appendix 3 Fig. S9). However, only one ASV, a fungus in the family Lasiosphaeriaceae (Ascomycota), was highly enriched in the Intermediate inoculum in *all* pairwise comparisons with the other four inocula.

DISCUSSION

Decades of research has highlighted the myriad ways soil microbes can affect the plants with which they interact. However, we know little about how spatial variation in soil microbial communities within and outside the distribution of native plant species influences plant fitness, local adaptation, and the location of geographic range limits. We used large reciprocal transplants of plant source populations and soils in the greenhouse and field to ask how variation in soil microbial communities affected *xantiana* lifetime fitness, whether plant populations were locally adapted to their home microbial communities, and whether the soil microbial communities outside *xantiana*'s geographic range margin may hinder colonization at those sites. We found strong spatial structure among microbial communities within and outside *xantiana*'s range, and this variation affected components of plant fitness in the greenhouse and field. Inoculum sourced from one site within the range positively affected components of fitness in both the greenhouse and field experiments, but *xantiana* populations were not adapted or maladapted to their local soil microbial communities. Pairing these results with sequencing of soil microbial communities suggests the potential for enemy release outside the range margin, but also that important mutualists contributing to *xantiana* fitness may be patchily distributed within and outside the subspecies' range.

Spatial variation in soil microbes contributes to fitness differences in the greenhouse and field

In the greenhouse, soil microbes from the Intermediate site increased growth relative to microbial communities from Center and Edge sites. Addition of inoculum from this same site, Intermediate, also increased a plant's probability of fruit production (given survival) by 25-50 percent in the second year of the field experiment. These results suggest that there are unique properties of the soil microbial community at the Intermediate site. Though enemy release is a plausible explanation for the increased fitness of plants growing with beyond-range inocula in the greenhouse (discussed below), if this were the mechanism driving increases in fitness associated with Intermediate inoculum, we would not expect the addition of Intermediate inoculum to beyond-range

sites in the field to improve components of fitness as it did. Rather, our results in aggregate indicate that there are soil mutualists present at the Intermediate site that are absent or in lower abundance at the other sites. Our sequencing of soil microbial communities used in the greenhouse experiment allows us to further explore how microbial compositional variation may relate to the observed experimental results.

Overall, the soil microbial community at the Intermediate site was most similar to other within-range sites, and dissimilar to beyond-range sites. Though all sites were dominated by Ascomycota and Basidiomycota, as might be expected, the Intermediate site had the highest relative proportion of reads belonging to the Glomeromycota, the arbuscular mycorrhizal fungi (AMF). AMF are among the globally most important soil mutualists for plants (van der Heijden *et al.*, 1998) and associate with over 80% of plant taxa, including *xantiana* (J. Benning, pers. obs). AMF have been shown to mediate a variety of environmental stressors for their plant hosts, including drought (Augé, 2001), herbivory (Gehring & Whitham, 2003), and nutrient limitation (Johnson *et al.*, 2010). Inoculation with Intermediate soil may have introduced relatively more AMF and increased the potential for root colonization by these mutualists, which could be especially important in the relatively stressful environments at and beyond *xantiana*'s range margin. Analyses of differential abundance for individual ASVs also highlighted one ASV unique to the Intermediate site which was particularly abundant there, a fungal taxon from the family Lasiosphaeriaceae. Though this family has traditionally been characterized as saprotrophs, amplicon sequencing studies are increasingly finding these fungi living as endophytes within plant roots or in rhizosphere soil (Su *et al.*, 2010; Tian *et al.*, 2018; Hugoni *et al.*, 2018). One recent such study found that a member of Lasiosphaeriaceae had large growth-promoting effects in the liverwort *Marchantia* (Nelson, 2017). We are currently sequencing roots and rhizosphere soil from the greenhouse experiment, and these additional data will shed further light on the rhizosphere-associated microbes driving fitness differences among inocula.

Geographic range limits

Could variation in microbial communities across *xantiana*'s range boundary affect the likelihood of population colonization outside that range margin? In the greenhouse, plants grown with beyond-range inocula were larger than those grown with Center and Edge inocula, and roughly on par with plants grown with Intermediate inocula. In addition, microbial communities beyond the range edge contained fewer taxa, had modestly reduced diversity, and had fewer unique taxa relative to communities from inside the range. Together, these results suggest the potential for release from microbial enemies outside *xantiana*'s range edge. Although pathogen loads may be smaller outside *xantiana*'s range edge, inoculation of beyond-range transplants with soil from the Intermediate site still substantially increased probability of fruiting, suggesting that beneficial microbes patchily distributed within *xantiana*'s range may be absent outside its range limit.

Though experimental work is scarce, a handful of studies have demonstrated the potential for the limited distribution of mutualists to restrict plant species' geographic ranges (Stanton-Geddes & Anderson, 2011; Moeller *et al.*, 2012; Afkhami *et al.*, 2014). It is unclear what is driving the distribution of microbial symbionts across and beyond *xantiana*'s range, though the decrease in ASV richness outside the range does correlate with an increase in aridity, a pattern also reflected in global analyses of fungal biogeography (Tedersoo *et al.*, 2014), but interestingly, not bacterial biogeography (Fierer & Jackson, 2006). Regardless of the underlying mechanisms, a patchy distribution of microbial mutualists within *xantiana*'s range decreases the likelihood of these mutualists dispersing beyond *xantiana*'s range limit. What will matter most for microbial colonization outside *xantiana*'s range limit is likely to be their abundance near that limit, and the fact that the largest differences among sources of inocula were between Intermediate and Edge soils raises the possibility that range edge habitat may be especially depauperate in microbial mutualists. In this case, *xantiana* individuals dispersing outside the range limit may be unlikely to encounter potentially important soil mutualists.

Though the benefits of inoculation with Intermediate soil in year 2 were not realized as increases in mean *lifetime* fitness (here, a proxy for population growth), the potential for depauperate mutualist communities outside the range margin to contribute to *xantiana*'s range limit should not be discounted. Precipitation in year 2 of the field experiment was considerably above average, and benefits derived from positive plant-microbe interactions may be more significant in more average (here, arid) abiotic conditions. Furthermore, in this experiment we did not manipulate the entire soil environment that plants experienced, but rather added a small amount of inoculum on top of the existing soil, which still had measurable effects on a component of plant fitness. Though logistically difficult, transferring entire soil cores from inside the range to outside would be a stronger test of how edaphic factors may limit *xantiana*'s distribution, and we have these experiments underway.

Local adaptation and maladaptation inside the range

There was no strong evidence that plant populations were locally adapted to their home soil microbial communities within the range. Local adaptation of plant populations to their soil biotic communities may be rare due to the accumulation of specialized microbial pathogens within sites (sensu Janzen, 1970), and the fact that differences in generation time between plants and their microbial pathogens may often tip the balance of any coevolutionary arms race toward rapidly reproducing microbes. However, in this study *xantiana* populations did not appear to be maladapted to their local microbial pools, either. Because microbial communities consist of thousands of distinct microbial populations spanning the parasite-mutualist continuum, clear patterns in adaptation of plant populations to their *overall* microbial community may be rare (Thrall *et al.*, 2007; Biere & Verhoeven, 2008; Lankau & Keymer, 2018). Interestingly, in the greenhouse experiment the smallest plants were those grown with sterilized control inoculum. This could result from adaptation of *xantiana* to its *regional* microbial community such that sterile soil is lacking in important generalist mutualists present across and beyond *xantiana*'s distribution in Southern California.

Conclusion

Although the phenotypic effects of microbes on plants have been studied for many decades, we are largely in the dark as to how plant-microbe interactions shape large scale patterns of plant diversity. Here we have shown that in greenhouse and field experiments, spatial variation in soil microbial communities affects plant fitness in ways that could potentially influence the geographic distribution of a native plant. When paired with characterization of microbial communities, experimental approaches, especially in the field, can offer much insight into the ecology and evolution of plant-microbe interactions. A greater understanding of aboveground-belowground interactions is needed to accurately forecast species distributions and manage ecosystems in the novel environments of the future as climate change, land use, and habitat fragmentation reshape landscapes globally (Van der Putten, 2012).

Chapter 4

Testing the influence of climatic and edaphic factors on lifetime fitness outside the geographic range margin of *Clarkia xantiana* ssp. *xantiana*

ABSTRACT

Plant species' distributions are often thought to overwhelmingly reflect their climatic niches. However, climate represents only a fraction of the *n*-dimensional environment to which plant populations adapt, and studies are increasingly uncovering strong effects of non-climatic factors on species' distributions. Here we use an intensive manipulative experiment to quantify the effects of precipitation and edaphic environment on plant fitness outside the geographic range boundary of a native California annual plant, whose distributional limit is associated with increased aridity. We grew plants outside the range edge in large mesocosms filled with soil either from within or outside its range and factorially manipulated precipitation. Across two years, edaphic environment had large effects on plant lifetime fitness that were similar in magnitude to the effects of precipitation. Moreover, mean fitness of plants grown with within-range soil in the low-water treatment approximated that of plants grown with beyond-range soil in the high-water treatment. The positive effects of within-range soil persisted in year 2, when natural precipitation was not limiting. These results are among the first to directly quantify the effects of edaphic variation on a plant species' range limit and highlight the need to include factors other than climate in models of species' distributions.

INTRODUCTION

Species' geographic range limits, the perimetric lines that delimit taxa's geographic distributions, are simple patterns resulting from the complex interplay of genetics, ecological interactions, and demography. For terrestrial plants, the most oft assumed drivers of geographic distributions are temperature and precipitation, and these climatic variables underlie most species distribution models (SDMs) (Pearson & Dawson, 2003; Sexton *et al.*, 2009; Louthan *et al.*, 2015). However, it is increasingly recognized that non-climatic factors likely play underappreciated roles in limiting species' large scale distributions (Parker, 2001; Araújo & Luoto, 2007; Sexton *et al.*, 2009; Gravel *et al.*, 2011; Bertrand *et al.*, 2012; Louthan *et al.*, 2015; Staniczenko *et al.*, 2018; Benning *et al.*, 2019). One environmental variable that is potentially pivotal in influencing plant species' distributions is the edaphic (soil) environment in which almost all terrestrial plants complete their life cycle (Bertrand *et al.*, 2012; Thuiller, 2013; Diekmann *et al.*, 2015). However, the role of plant - soil interactions in modulating large scale plant distributions remains sorely understudied.

Soils exhibit remarkable diversity in biotic (Fierer & Jackson, 2006; Tedersoo *et al.*, 2014) and abiotic (Palm *et al.*, 2007) properties, and these vary at both small and large scales. It is widely recognized that variation in the hyperdimensional soil environment can drive ecosystem level patterns in productivity and terrestrial community composition (Ettema & Wardle, 2002; Zak *et al.*, 2003; Güsewell, 2004; Palm *et al.*, 2007; Van Der Heijden *et al.*, 2008; Wubs *et al.*, 2019), and that biotic (Lugtenberg & Kamilova, 2009; Miransari, 2010; Hayat *et al.*, 2010; Jung *et al.*, 2012) and abiotic (Passioura, 1991; Mengel *et al.*, 2001) components of soil have a range of potentially large effects on individual plant phenotypes and fitness. However, the potential role edaphic factors play in the colonization of, and fitness within, novel environments outside a taxon's range limit has received surprisingly little attention (but see Nuñez *et al.*, 2009; Peay *et al.*, 2010; Stanton-Geddes et al. 2012; Brown & Vellend, 2014; Osborne *et al.*, 2018).

Given the demonstrated effects of soil microbial communities on plant growth (Keymer & Lankau, 2017; Pain *et al.*, 2018), phenology (Lau & Lennon, 2012; Wagner

et al., 2014), defense (Bennett *et al.*, 2009; Jung *et al.*, 2012), and reproduction (Wolfe *et al.*, 2005), the ubiquity of plant - soil microbe interactions, and the high spatial turnover of soil microbial communities (Noguez *et al.*, 2005; Wolfe *et al.*, 2007; Prober *et al.*, 2015; Maestre *et al.*, 2015), plant - soil microbe interactions could play an underappreciated role in modulating plant distributions (Parker, 2001; Van der Putten *et al.*, 2010; Van der Putten, 2012). If compatible microbial mutualists are absent, or novel pathogens present, outside a plant species' range limit, novel soil microbial assemblages could impair fitness outside that limit (Peay *et al.*, 2010; Brown & Vellend, 2014; Lankau & Keymer, 2016). Presence of mutualists may be especially important for ameliorating increased abiotic stress that often occurs at and beyond range margins, like that experienced by plants distributed across precipitation gradients. For example, although the mechanisms are still opaque, there is mounting evidence that soil microbes can alleviate water stress for plants (Augé, 2001; Yang *et al.*, 2009; Miransari, 2010; Lau & Lennon, 2012).

Variation in abiotic properties of soil, such as nutrient and organic matter content, across a species' distributional boundary could also influence the potential for population colonization outside the range. Including abiotic edaphic factors in SDMs often improves their performance over climate-only models (Bertrand *et al.*, 2012; Dubuis *et al.*, 2013; Walthert & Meier, 2017), suggesting that these factors play roles in structuring plant species' geographic distributions. Abiotic edaphic factors are known to structure local / population range limits, like those of plants adapted to serpentine soils (Lau *et al.*, 2008; Lazarus *et al.*, 2011), but experiments testing their potential role in constraining fitness outside a geographic range margin are scarce (but see Stanton-Geddes *et al.*, 2012; Brown & Vellend, 2014).

Manipulative transplant experiments let us ask directly, what limits lifetime fitness and prevents range expansion beyond a taxon's current geographic range limit? Factorial experiments manipulating multiple putatively important environmental variables also let us assess the *relative* magnitude of these effects, and potential interactions betwixt them, in a way that correlative approaches cannot. We designed an experiment to disentangle the role of edaphic environment and precipitation in range

adaptation of a California annual plant by experimentally manipulating these two niche variables in a transplant experiment beyond the plant's geographic range boundary. The plant, *Clarkia xantiana* ssp. *xantiana*, is distributed across an aridity gradient, with lower precipitation near and outside its range edge, and SDMs indicate this aridity gradient is strongly correlated with the subspecies' distribution (Eckhart *et al.*, 2011). We quantified the relative, and potentially interactive effects of edaphic environment and precipitation on *C. x. xantiana* lifetime fitness outside this range boundary in the first year of the experiment. In the second year of the experiment when natural precipitation was well above average, we did not manipulate precipitation but rather focused solely on the effects of edaphic environment on *C. x. xantiana* fitness outside its range edge.

METHODS

Study System

Clarkia xantiana ssp. *xantiana* A. Gray (hereafter, *xantiana*) is a winter annual native to the Southern Sierra Nevada foothills of California (USA). *Xantiana* is most commonly found on steep slopes at low to intermediate elevations (500 - 1500 m) in grasslands, oak and pine woodlands, and openings in chaparral (Lewis & Lewis, 1955; Eckhart & Geber, 1999). The subspecies is distributed between California's Central Valley to the west and the Mojave Desert to the east, with the greatest density of populations occurring within the Kern River Valley. Most populations occur on sandy, fast draining soils derived from igneous rock (granodiorite, granite, quartz monzonite, and/or gabbro) (Eckhart *et al.*, 2010). In the Mediterranean climate of the Southern Sierra Nevada, *xantiana* germinates in the relatively wet winter, maturing and setting seed in June.

Xantiana's eastern range edge is stark (Fig. 4.1a) and extensive searching over the past 20+ years has uncovered no *xantiana* populations beyond this limit. *Xantiana*'s sister subspecies, the largely selfing *C. x. parviflora*, is distributed mainly to the east of *xantiana*, and the two taxa are in secondary contact within a narrow (ca. 10 km) zone of sympatry at *xantiana*'s eastern (and *C. x. parviflora*'s western) range edge (Pettengill & Moeller, 2012; Briscoe Runquist *et al.*, 2014).

Xantiana is distributed across a west-east aridity gradient (with precipitation lower and more variable toward and outside its eastern range edge) that contributes to reduced performance at the range edge and beyond (Fig. 4.1; Eckhart *et al.*, 2010, 2011). In addition, mutualistic interactions with pollinators are weaker at and beyond the range limit, resulting in greater pollen limitation of reproduction (Moeller *et al.*, 2012; Anderson *et al.*, 2015), and antagonistic interactions with mammalian herbivores are stronger at and beyond the range limit (Benning et al. 2019).

Overview of Experimental Design

We conducted the experiment at a site 4 km beyond *xantiana*'s eastern range edge that hosts a population of *C. x. parviflora* (Site 66; Fig. 4.1a). In order to manipulate edaphic environments and water availability, we installed 160 mesocosms in 20 blocks across a large slope (ca. 100 x 30 m) where all natural vegetation was allowed to remain intact. The field site is an arid shrubland dominated by sagebrush (*Artemisia tridentata*) and longspine horsebrush (*Tetradymia axillaris* var. *longispina*), and sparse annual vegetation. The mesocosms were filled with soil from one of two sites: the focal site (Site 66), 4 km outside *xantiana*'s eastern range edge, or from a site 18 km southwest, 11 km west of *xantiana*'s eastern range edge, that hosts a natural *xantiana* population (Borel Road). All mesocosms were planted with seeds from the same within-range population (Borel Road). Both the beyond-range experimental site and the within-range source population occur on fast draining soils derived from Mesozoic granite.

Mesocosm Construction and Installation

We based our mesocosm design on the in-growth cores of Johnson et al. (2001), with some modifications. Each mesocosm was constructed from 70 cm long sections of PVC pipe (10 cm interior diameter; 11.5 cm exterior diameter) in order to afford plants a large belowground environment that allows for natural root growth (Fig. 4.1b,c). One end of the section was capped with PVC caps, which were glued to the sections using PVC glue. To allow water movement across the mesocosm exterior, we cut two openings (32 × 5 cm) into the sides of each mesocosm, and one large hole (8 cm diameter) into the

bottom cap. These slits and holes were covered with 0.5 micron nylon mesh (Plastok Associates, Ltd., England), which was attached to the mesocosm with an elastic adhesive (Lexel; Sashco, Brighton, Colorado, USA). This mesh kept *xantiana* roots from growing outside the mesocosms and should prevent most fungi (Teste *et al.*, 2006; Islam *et al.*, 2017) and many bacteria (Levin & Angert, 2015) from entering the mesocosms from the surrounding soil environment. We installed mesocosms at the site in October 2017. We prepared holes for each mesocosm using an auger powered by a portable drill, and set mesocosms into the hole such that no more than 3 cm of the mesocosm protruded above the soil surface. We tamped soil around perimeter around each mesocosm to ensure contact between the mesocosm and the surrounding soil.

We collected soil from the focal site, and the seed source site, to fill the mesocosms. Soil was collected from the top 60 cm at multiple locations within the natural *xantiana* population at Borel Road, and from multiple locations within the transplant site (> 10 locations per site). Soil was manually homogenized on tarps. We filled mesocosms with ca. 5 L of soil each; there were 80 mesocosms in each within and beyond-range soil treatment (N = 160). Soil and watering treatments were assigned randomly across 20 blocks within the site using a complete randomized block design (watering n = 80). All mesocosms were caged to protect plants from mammal herbivory, which can be very common in this region (Benning *et al.*, 2019). Despite this effort, some cages were breached by small rodents; any plants that were eaten were removed from analyses below.

The experiment included control mesocosms (n = 20 of each soil type) which were filled with soil that had been autoclaved twice for 1 hr, with a 4 hr rest between autoclave cycles. The purpose of these controls was to parse abiotic vs. biotic edaphic effects. However, given the small number of plants that were available for analyses in these control groups (e.g., 1-5 fruiting plants per soil treatment × water treatment combination in year 1) and a lack of confidence that sterilization of the soil was indeed effective, we have excluded them from the analyses below. Forthcoming molecular analyses of microbial communities in live and control mesocosm soils will allow us to determine the efficacy of our sterilization effort.

Sowing, Watering Treatment, and Phenotyping

Year 1 In October 2017, 15 seeds were sown into each pot. Because conditions were so dry and germination did not occur in all mesocosms, we added an additional 10 seeds to mesocosms that had fewer than 2 germinants in February (n = 128 mesocosms). These varying seed numbers are accounted for in analyses of germination below. Despite the seed addition, only 18 mesocosms that did not have germinants in February had seedlings in April.

Because precipitation during the 2017-2018 winter was well below average (Fig. 4.2), we watered mesocosms monthly in December, January, and February to promote germination and maximize sample sizes. Each monthly watering added ca. 525 mL of water to each mesocosm. In March, when plants begin to grow quickly aboveground, we began the watering treatment. All low water mesocosms received ca. 175 mL of water and no supplemental water thereafter, and high-water mesocosms received ca. 525 mL once in March, April, and May. All watering treatments were applied to mesocosms regardless of whether or not they contained germinants. We culled each mesocosm to one seedling in March. We recorded germination in February, March, and April, and survival, height, and the number of flower buds in May. We collected all fruits from each plant in late June. Soil volumetric water content (VWC) was measured with a Decagon GS1 soil moisture meter (METER Group, USA) in a subset of mesocosms in April and May, ca. 24 hr after imposing the watering treatment.

Year 2 We reseeded all pots with an additional 15 seeds (collected in June 2018) in October 2018. We did not include a watering treatment in year 2. We recorded germination in February and culled each mesocosm to one seedling. We recorded survival, height, and the number of flower buds in May, and collected all fruits from each plant in late June. Soil VWC was measured with a Decagon GS1 soil moisture meter (METER Group, USA) in a subset of mesocosms in February and May. A subset of mesocosms (n = 42) were removed in May to harvest soil for nutrient analyses, and rhizosphere and roots for molecular analyses of microbial communities. Using data from the remaining mesocosms, we predicted final seed set for removed mesocosms using a linear model of seed set given number of flower buds in May. Number of buds in May

was highly correlated with final seed set (r = 0.81; model adjusted R^2 = 0.66). We used these predicted values in the analyses below to retain these individuals in the data; analyses omitting these removed mesocosms produced similar results. Soil from these removed mesocosms was also used for analyses of soil pH, macro- (nitrate, phosphorus, potassium, calcium, magnesium, and sulfur) and micro-nutrient (aluminum, boron, iron, manganese, lead, and zinc) content, soil organic matter (SOM), and estimated cation exchange capacity (CEC); all soil analyses were performed at the University of Connecticut Soil Nutrient Analysis Laboratory. Soil nutrients were quantified using a modified-Morgan solution, SOM was determined by loss on ignition, and CEC was estimated with the summation method based on levels of Ca, Mg, and K.

We used the PRISM climate dataset (PRISM Climate Group) to obtain interpolated estimates of monthly precipitation data for each site during the two years of the experiment, at 4 km grid cell resolution. We also obtained PRISM precipitation records for the site in years 1991 - 2017, in order to interpret precipitation patterns during the experiment relative to long-term trends.

Statistical Analyses

All analyses were conducted in R (R Core Team 2013). We used *aster* life history models (Geyer *et al.*, 2007; Shaw *et al.*, 2008) in R (R Core Team, 2013) to evaluate the effects of soil environment and water treatments on *xantiana* lifetime fitness. *Aster* models use a graphical approach that links sequential components of lifetime fitness, each modeled with its appropriate statistical distribution. Our *aster* model incorporated four components of lifetime fitness (*nodes* in the graphical model) for this experiment: germination, early survival (March), fruit production (i.e., did the plant produce any seed-bearing fruits), and total seed set. The first three components were modeled as Bernoulli variables (0,1), and total seed set was modeled as a zero-truncated negative binomial variable:

$$\mathbf{1} \rightarrow \underset{(0,1)\ Bernoulli}{\boldsymbol{germination}} \rightarrow \underset{(0,1)\ Bernoulli}{\boldsymbol{early\ survival}} \rightarrow \underset{(0,1)\ Bernoulli}{\boldsymbol{fruit\ production}} \rightarrow \underset{zero-truncated\ negative\ binomial}{\boldsymbol{seeds\ produced}}$$

For year 1, we built an *aster* model with soil treatment, water treatment, and their interaction as predictors; response variables were those associated with each component of lifetime fitness. To estimate the effects of each predictor on lifetime fitness, each predictor was fit at the level of total seed set in the model (Shaw *et al.*, 2008). We did not include block as a random effect in these models because random effects cannot be estimated in *aster* models that include nodes modeled with negative binomial distributions (C. Geyer, pers. comm.). We used likelihood ratio tests (LRTs) comparing submodels to fuller models to test each term of interest. For year 2, we modeled lifetime fitness as above but included only soil treatment as a predictor, since we did not impose a watering treatment in year 2, and confirmed via LRT of *aster* models that watering treatment during year 1 did not affect lifetime fitness in year 2.

Individual life history components Because effects of soil and water treatments may manifest at different stages of a plant's life, we also tested the influence of inoculum treatment at each life history stage separately. We used logistic regressions to test the effects of soil treatment, water treatment, and their interaction, on our Bernoulli life history components (germination through fruit production). Using the same model structure, we used negative binomial regression to model seed production. These separate fitness component analyses are "conditional" in the sense that, for each component after germination, we only used the subset of records that survived the previous life history stage (e.g., analysis of the probability of fruiting only included those plants that survived to March).

Predicting mean lifetime fitness The fitness metric of most interest was mean seeds produced per planted seed. Because we planted multiple seeds per mesocosm, and culled extra germinants, fitness predictions from our *aster* model that includes germination as a simple bernoulli variable (the mesocosm either contained germinant(s) or not) would be inflated relative to this metric. Thus, we obtained predicted values for lifetime fitness and their associated standard errors by taking the product of germination probabilities (see above) and unconditional parameter estimates from a full aster model that did not include a germination node. Standard errors for these products were calculated using the Delta method (Buehler, 1957).

Soil nutrient and VWC analyses We used Student's t-tests to test for differences in soil pH, macro- and micro-nutrient content, soil organic matter, and CEC of the within- and beyond-range soils in the mesocosms exhumed in year 2. To determine if differences in these abiotic soil variables were related to fitness differences between soil treatments, we used linear regression to test the effects of each variable on plant height in May (which is highly correlated with final seed set; $r = 0.72$). To quantify differences in VWC between watering treatments and soil types in year 1, we built a linear model with VWC as the response and soil treatment, water treatment, the soil × water interaction, and sampling month (April or May) as predictors. To quantify differences in VWC between soil types in year 2, we built a linear model with VWC as the response and soil treatment and sampling month (February or May) as predictors.

RESULTS

Year 1

During the year 1 growing season, the beyond range transplant site received 154.3 mm of rain (57% of the 29 yr average of 270.0 mm; Fig. 4.2). There was at least one germinant in 104 mesocosms (76%), and a fruiting plant in 78 mesocosms (57%). Both soil and water treatment had large effects on lifetime fitness as modeled by *aster* analyses (both $P < 0.001$), but there was no support for an interactive effect of the two treatments (Table 4.1; Fig. 4.3). Overall, within-range soil increased lifetime fitness ca. 170% relative to plants grown with beyond-range soil (22.6 vs 8.3 seeds per planted seed, respectively). Plants in the high water treatment had lifetime fitness values ca. 250% higher than those plants in the low water treatment (24.0 vs. 6.8 seeds per planted seed, respectively). Interestingly, lifetime fitness of plants grown with beyond-range soil and high water was not significantly different from that of plants grown with within-range soil and low water (13.1 vs. 10.2 seeds per planted seed, respectively; LR dev = 1.4, $P = 0.2$; Fig. 4.3).

The effects of soil and watering treatments varied among life history stages. Watering treatment had no effect on late survival or probability of fruiting, but increased watering resulted in a more than 200% increase in seed set (351 vs. 110 seeds; Table 4.2;

Fig. 4.3). Soil from within range increased probability of germination (within-range: 0.09, beyond-range: 0.06) and late survival (0.94 vs. 0.77), as well as seed set (251 vs. 155) relative to soil from beyond range.

Year 2

In year 2, the transplant site received 412.5 mm of rain, 52% more than the long term average (270.0 mm; Fig. 4.2). There was at least one germinant in 150 mesocosms (96%), and a fruiting plant in 116 mesocosms (74%). Average lifetime fitness was more than twice as high as in year 1 (37.7 vs. 15.4 seeds per planted seed, respectively; Fig. 4.3). Soil treatment had a more modest, and variable, effect on lifetime fitness than in year 1, but in the same direction: within-range soil increased lifetime fitness ca. 30% relative to beyond-range soil (42.7 vs 32.6 seeds per planted seed, respectively; Table 4.1, Fig. 4.3). Soil treatment affected both probability of germination and seed set (Table 4.2), but in opposite directions: within-range soil decreased probability of germination by 14% (within-range: 0.19 vs. beyond-range: 0.22), but increased seed set by 60% (295.3 vs. 182.9 seeds, respectively) relative to beyond-range soil. Year 1 watering treatment and whether or not a mesocosm contained a plant in year 1 did not affect lifetime fitness in mesocosms in year 2.

Soil nutrient and water content

Though both soils were, overall, nutrient poor, within-range soil was more nutrient rich than beyond-range soil, had a slightly lower pH, higher SOM, and higher CEC (Appendix 4 Fig. S1). The largest significant differences in nutrient content between within and beyond-range soils were for magnesium (108.0 vs. 55.2 ppm, respectively), calcium (1040.3 vs. 554.0 ppm), sulfur (20.4 vs. 11.0 ppm), and nitrate (4.0 vs 1.5 ppm). CEC of within-range soil was double that of beyond-range soil (7.0 vs 3.5 cmole+ / 100g, respectively), and within-range soil had higher SOM than beyond-range soil (1.1 vs. 0.2 percent, respectively). Plant height was significantly positively correlated with mesocosm soil CEC ($P = 0.01$), calcium ($P < 0.01$), magnesium ($P < 0.01$), sulfur ($P = 0.02$), and nitrate ($P < 0.01$) (Appendix 4 Fig. S2). The relationship of height with nitrate was driven

by one within-range soil outlier with a nitrate level 47% higher than the next highest nitrate record; when this outlier was removed, the relationship between height and nitrate was not significant ($P = 0.2$).

In year 1, watering increased mesocosm VWC (Θ) from 0.02 to 0.15, averaging across soil types and measurements in April and May. (Note that measurements were taken ca. 24 hr after each monthly watering treatment, and that soil VWC likely equilibrated between treatments rather quickly afterward.) VWC also differed between within and beyond-range soil, though differences were relatively small. VWC in mesocosms with within-range soil was higher after watering relative to beyond-range soil (0.16 vs. 0.13; Tukey $P = 0.05$); there was no difference in VWC between soil types in the low water treatment. In year 2, when there was no watering treatment, VWC in mesocosms with within-range soil was again higher than VWC in mesocosms with beyond-range soil (0.15 vs. 0.13, respectively; $P < 0.01$), averaging across 21 measurements in February and May.

DISCUSSION

The question of why, in the absence of obvious barriers, an organism occurs on one side of its range margin and not the other is a perennial one. In many cases, the simple (and unsatisfactory) answer is that the organism is adapted to environments inside its range, and maladapted to the environment outside its range. If we are to more accurately predict future species' distributions and understand the limits of adaptation, we must go beyond correlative models and test specific hypotheses regarding the environmental factors underlying maladaptation and restricting range limits, preferably conducting these tests with experiments in the field. If factors other than climate have a large influence on a given species' distribution, predictions from climatic SDMs are likely to be poor. Using an intensive manipulative field experiment across two years, we have provided direct evidence that lifetime fitness of transplants outside a native plant's distribution is strongly limited by edaphic factors, that these effects are large, and that they are similar in magnitude to those caused by the putatively overarching climatic driver, precipitation.

In year 1, both soil and water treatments had large effects on lifetime fitness, with increased watering and within-range soil each more than doubling mean lifetime fitness in *xantiana* growing outside its range limit. The precipitation gradient across which *xantiana* is distributed is well characterized (Eckhart *et al.*, 2010, 2011), and the first year of this experiment confirmed experimentally what correlative approaches have previously suggested; i.e., that decreased precipitation limits fitness outside *xantiana*'s range margin. What is more surprising is that soil moved from just 18 km southwest of the beyond-range transplant site more than doubled lifetime fitness in *xantiana* planted outside its range. Calls to explore the potential for edaphic factors to limit large scale plant distributions (Lafleur *et al.*, 2010; Thuiller, 2013; Diekmann *et al.*, 2015) have been answered to some extent by incorporating soil variables into SDMs (Bertrand *et al.*, 2012; Dubuis *et al.*, 2013; Beauregard & de Blois, 2014; Walthert & Meier, 2017; Buri *et al.*, 2017), and edaphic factors often improve the fit of these models relative to models fit only with climatic variables. However, experimental investigations are lacking (but see Stanton-Geddes *et al.*, 2012; Brown & Vellend, 2014), and to our knowledge, ours is the first to directly quantify the effect of edaphic variation across a geographic range boundary on plant lifetime fitness outside that boundary. The magnitude of this effect driven by two soils that are geographically proximate and relatively similar in abiotic properties provides strong evidence that edaphic factors deserve much greater attention in explorations of plant species' range limits.

Beyond the overall fitness advantage conferred by within-range soil, two observations in particular are of note: first is the contrast between beyond-range soil with high water, and within-range soil with low water — lifetime fitness estimates within these two treatment combinations were not significantly different from each other. Second, in the low water treatment, which produced extremely low levels of soil moisture, within-range soil increased fitness ca. 200% relative to beyond-range soil. These results suggest that edaphic factors can mediate stressful conditions resulting from low precipitation outside *xantiana*'s range. There is increasing evidence that soil microbial communities can alleviate drought stress in plants (Augé, 2001; Lau & Lennon, 2012; Gehring *et al.*, 2017; Fitzpatrick *et al.*, 2019), and *xantiana* may benefit from

interactions with soil microbes from within its range that are absent or less abundant outside its range limit. Another set of experiments, detailed in Chapter 3 of this dissertation, indicated that soil microbial communities from one site within *xantiana*'s range increased components of *xantiana* lifetime fitness in both greenhouse and field experiments; this site was less than 4 km from the within-range soil source in the current study (Borel Road). Increased mineral nutrition of plants growing in within-range soil could also contribute to increased drought tolerance, either through direct mechanisms modulated by specific nutrients or simply due to overall increased nutrition, especially if this results in larger root systems (Waraich *et al.*, 2011; Ahanger *et al.*, 2016).

In year 2, when precipitation was ca. 50% higher than the long-term average, soil effects were more subtle. But despite being released from limiting precipitation, *xantiana* seed set was still improved ca. 60%, and lifetime fitness ca. 30%, when grown with soil from within its range. This highlights how the relative importance of environmental variables for regulating plant population dynamics can change over time, and that identification of constraints on species' distributions greatly benefits from multi-year studies. For instance, the effects of mammalian herbivory in this system are severe in years of higher rainfall, but minimal in low rainfall years (Benning et al. 2019). Temporal variation in the current study also affected the life stages at which soil treatment effects were realized. In year 1, within-range soil positively affected germination, late survival, and seed set. Due to increased rainfall in year 2, plant survival after germination was high in all treatments, and all positive effects of within-range soil were realized at seed set. Interestingly, the effects of soil treatment on seed germination was reversed in year 2, when germination rates were higher in beyond-range soil. It is difficult to know the mechanism underlying this pattern, but it seems somewhat unlikely that soil nutrient differences would have effects at this early life history stage, and presumably the direction of those effects would not be dependent on precipitation. On the other hand, soil microbes can affect seed germination rates and early survival (Gallery *et al.*, 2007; Sarmiento *et al.*, 2017; Osborne *et al.*, 2018), and their effects are very likely to be dependent on environmental context (Johnson *et al.*, 1997; Hoeksema *et al.*, 2010). While mutualistic soil microbial species may have increased germination in within-range soil in

year 1, increased rainfall in year 2 could have favored more growth of pathogenic microbial species.

Soil abiotic differences

The effects of edaphic environment on *xantiana* fitness are likely due to a combination of biotic and abiotic properties differing between the soils. Analyses of soil microbial communities in within- and beyond-range soils are forthcoming and will aid in determining the extent to which plant - soil microbe interactions contribute to this fitness variation. In the meantime, with the data currently at hand we can conjecture as to which abiotic soil variables may play important roles.

Both soils used in the experiment are derived from the same parent material (Mesozoic granite), are fast draining with little clay content, and can be considered "nutrient poor," but they did differ in some abiotic properties. Within-range soil seemed to have slightly greater water holding capacity (as indicated by higher soil VWC), likely due to its higher SOM content relative to beyond-range soil. Differences in SOM between these soils is to be expected given the gradient in primary productivity that separates the sites from which they are sourced (Fig. 4.1a). However, we feel it is unlikely that the large differences in fitness between soil types is primarily due to their relatively small difference in soil VWC, especially given that fitness differences persisted in year 2, when water was not likely limiting. Within-range soil was also more fertile, though only a subset of soil variables were significantly associated with differences in plant height. Interestingly, these were the secondary nutrients calcium (Ca), magnesium (Mg), and sulfur (S), all of which are strongly related to soil SOM in most soils.

Though these secondary nutrients (Ca, Mg, and S) receive less attention than N, P, and K for their roles in plant nutrition, they are all essential for plant growth and required in relatively large amounts. When we examined the relationship of Ca, Mg, and S with plant height and fit trend lines for each soil type separately (Appendix 4 Fig. S3), an interesting pattern emerged. For beyond-range soil, increasing amounts of these nutrients lead to increased plant height; however, there is no indication of a positive relationship with height for those plants grown with within-range soil. This suggests that,

if these nutrients are limiting, the relationship with plant growth might not be linear, but rather asymptotic — i.e., there is a threshold quantity of soil Ca, Mg, and S necessary for optimal *xantiana* growth, and that values above this threshold have little further effect on growth. This could indicate that this *xantiana* population is well adapted to the full range of Ca, Mg, and S values that occur in soils from within its source site, but maladapted to lower soil fertility levels that occur outside its range.

Niche variables will not shift synchronously with climate change

Research on species' geographic range limits is increasingly spurred by a desire to predict species' distributions under various scenarios of future climate change, usually using SDM's based on climatic variables. Often the underlying assumption in these models is that if populations' ranges shift along with their optimal climatic isotherms, their future distribution will reflect the future location of these climatic niche envelopes. However, unless all aspects of the *n*-dimensional environment shift synchronously, species will *always* encounter novel environments, to varying extents, with climate change, regardless of whether they disperse to new areas. Thus, determining the relative importance of various environmental variables for restricting a species' distribution is essential to accurately forecast the movement of geographic range limits.

There is no *a priori* reason to believe that environment variables will shift synchronously. There is little inherent limit on the rate of change of climatic variables like temperature and precipitation. However, other environmental axes such as belowground biotic and abiotic properties, may change much more slowly, if at all, with climate change (Van der Putten, 2012). For example, soil temperature responds more slowly than air temperature (Gehrig-Fasel *et al.*, 2008), soil nutrients often have limited responses to experimental temperature manipulations (Lamb *et al.*, 2011; Zamin *et al.*, 2014), and soil microbial communities may respond slowly to even sudden shifts in the abiotic environment (Waldrop & Firestone, 2006; Rinnan *et al.*, 2007; Cruz-Martínez *et al.*, 2009; Cregger *et al.*, 2012). When we disregard non-climatic factors in discussions of shifting species' distributions, we are implicitly collapsing an organism's *n*-dimensional niche down to just a few axes, and run the risk of greatly overestimating our ability to

accurately predict future site suitability across the landscape (Van der Putten *et al.*, 2010; Bertrand *et al.*, 2012). Further, if organisms within a habitat do not migrate or respond at the same rate as the climate changes, the complex web of species interactions within which every species is situated will change. There is mounting evidence that the decoupling of historical biotic interactions [e.g., herbivory (Visser & Holleman, 2001); competition (Alexander *et al.*, 2015)] will likely greatly complicate our predictions of species range shifts (Van der Putten *et al.*, 2010).

Conclusion

We often model distributions based on a very narrow subset of environmental axes, namely, temperature and precipitation. But organismal niches are hyperdimensional, and as researchers increasingly test for influences other than climate on species' large scale distributions, evidence for their importance accumulates. These observations hint at geographic range limits perhaps representing a "perfect storm" of maladaptation along multiple environmental (and trait) axes (*sensu* Antonovics, 1976). Manipulative experiments allow us to directly compare the relative magnitude of effects for putatively important environmental variables, and allow strong inference into current constraints on distributions. However, for predicting future species' distributions *en masse,* intensive experiments will not be logistically feasible. In those cases, natural history knowledge, population demography, mechanistic modeling, and increased environmental sampling of non-climatic variables will help improve forecasts. Likely the most important management concerns will be maintenance of large population sizes and high connectivity, as these will promote dispersal, adaptive gene flow, and *in situ* evolution in response to changing environments.

Figures – Introduction

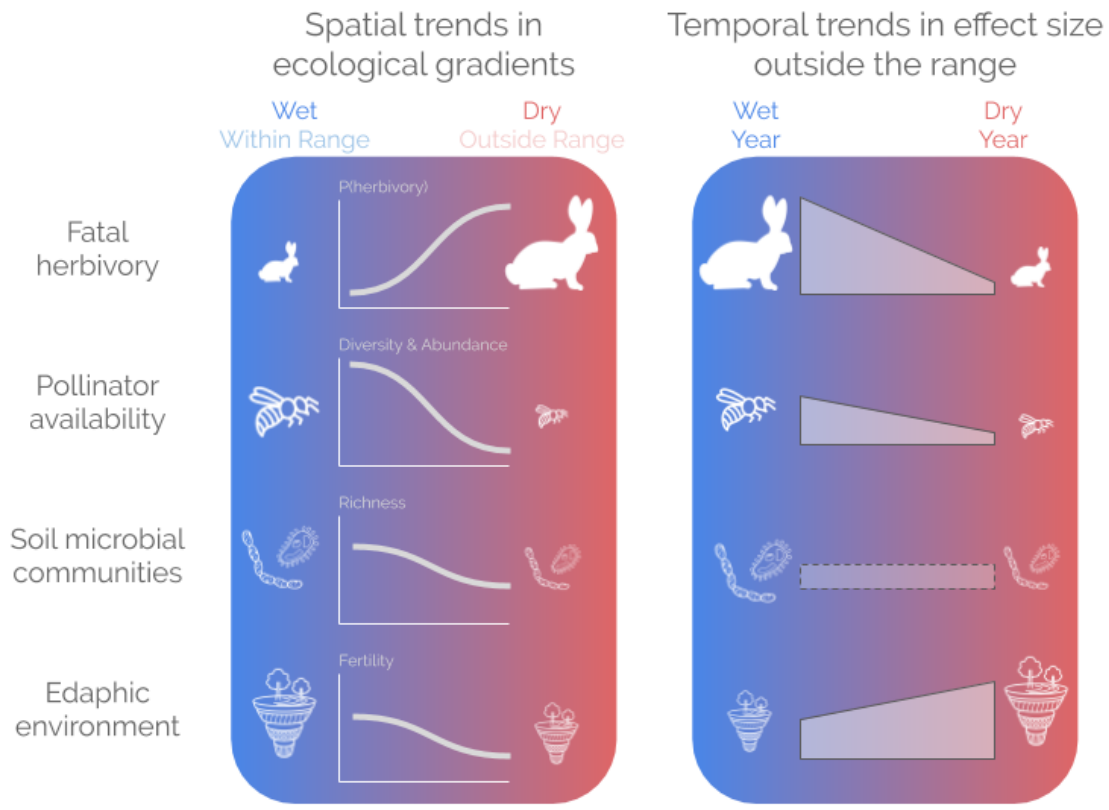

Figure I.1. Conceptual overview of dissertation, highlighting spatial trends in four ecological gradients across and beyond the range of *C. x. xantiana* (left), and temporal trends in the effect size of these environmental factors on plant lifetime fitness (right), across spatial and temporal gradients in precipitation. Left: Going from the center to outside *C. x. xantiana*'s distribution across an abiotic gradient of increasing aridity, probability of herbivory increases steeply (Chapters 1, 2), pollinator availability declines sharply (Chapter 2), soil microbial community richness declines moderately (Chapter 3), and edaphic conditions deteriorate (Chapter 4). Right: The magnitudes of effect of these ecological gradients on *C. x. xantiana* lifetime fitness outside its range margin often vary temporally between years of high and low precipitation: herbivory and pollen limitation influence fitness in relatively wet years when plant population sizes are substantial, but these effects are overwhelmed by limited water availability in dry years (Chapters 1, 2). More experiments are needed to determine if effects of soil microbial communities are contingent upon precipitation (Chapters 3, 4). Positive effects of within-range edaphic environment (including biotic and abiotic components) were stronger in the dry compared to the wet year (Chapter 4).

Figures – Chapter 1

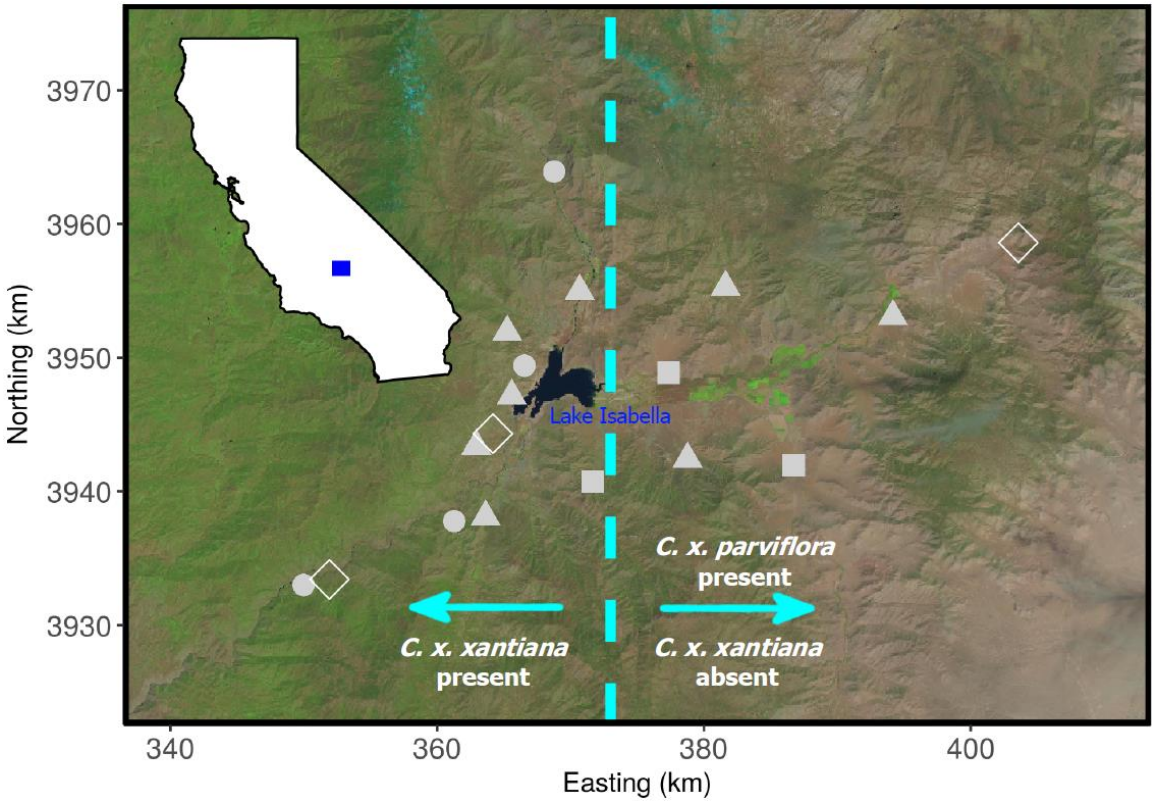

Figure 1.1. Geographic distribution of *Clarkia xantiana*, where the dashed blue line marks *C. x. xantiana*'s eastern range limit. The bulk of *C. x. parviflora*'s distribution lies east of this limit, though the two taxa share a narrow zone of sympatry around Lake Isabella. *C. x parviflora*'s western range edge is located near Easting 360. Points mark locations of stem translocation sites (2015, circles; 2016, triangles; both years, squares) and reciprocal transplant sites (diamonds). Background image is 19 April 2016 LANDSAT imagery of study area.

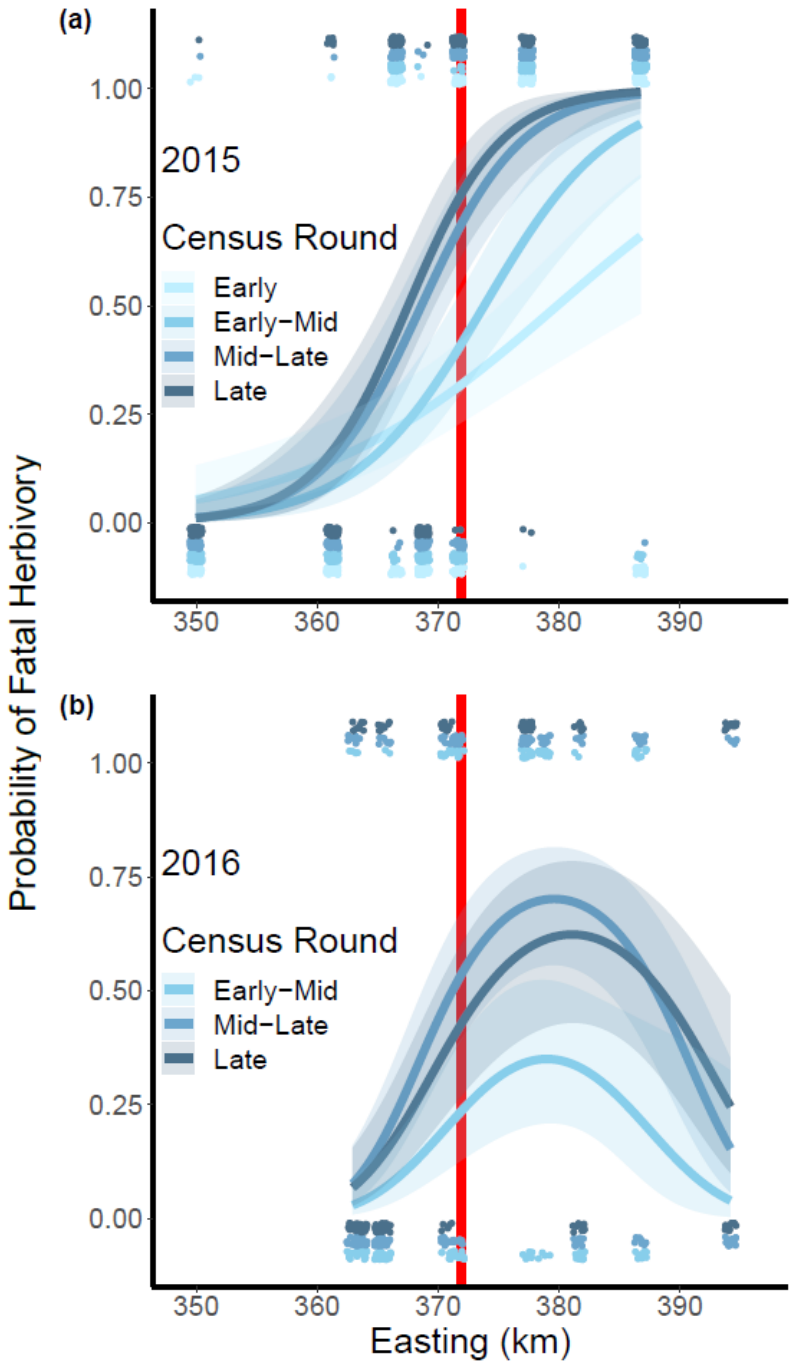

Figure 1.2. Spatio-temporal variation in probability of herbivory across and beyond *xantiana*'s range. The red line shows the location of *xantiana*'s eastern range limit. Plots show the relationship of probability of herbivory with easting and time (census round) from logistic regression for (a) 2015 and (b) 2016. For each plot, conditional effects of easting and time are shown, holding other model factors constant. Colors correspond to temporal replicates (ca. one replicate per week in June). Jittered points show individual plants, which either did or did not experience herbivory. Ribbons show 95% confidence bands for predictions.

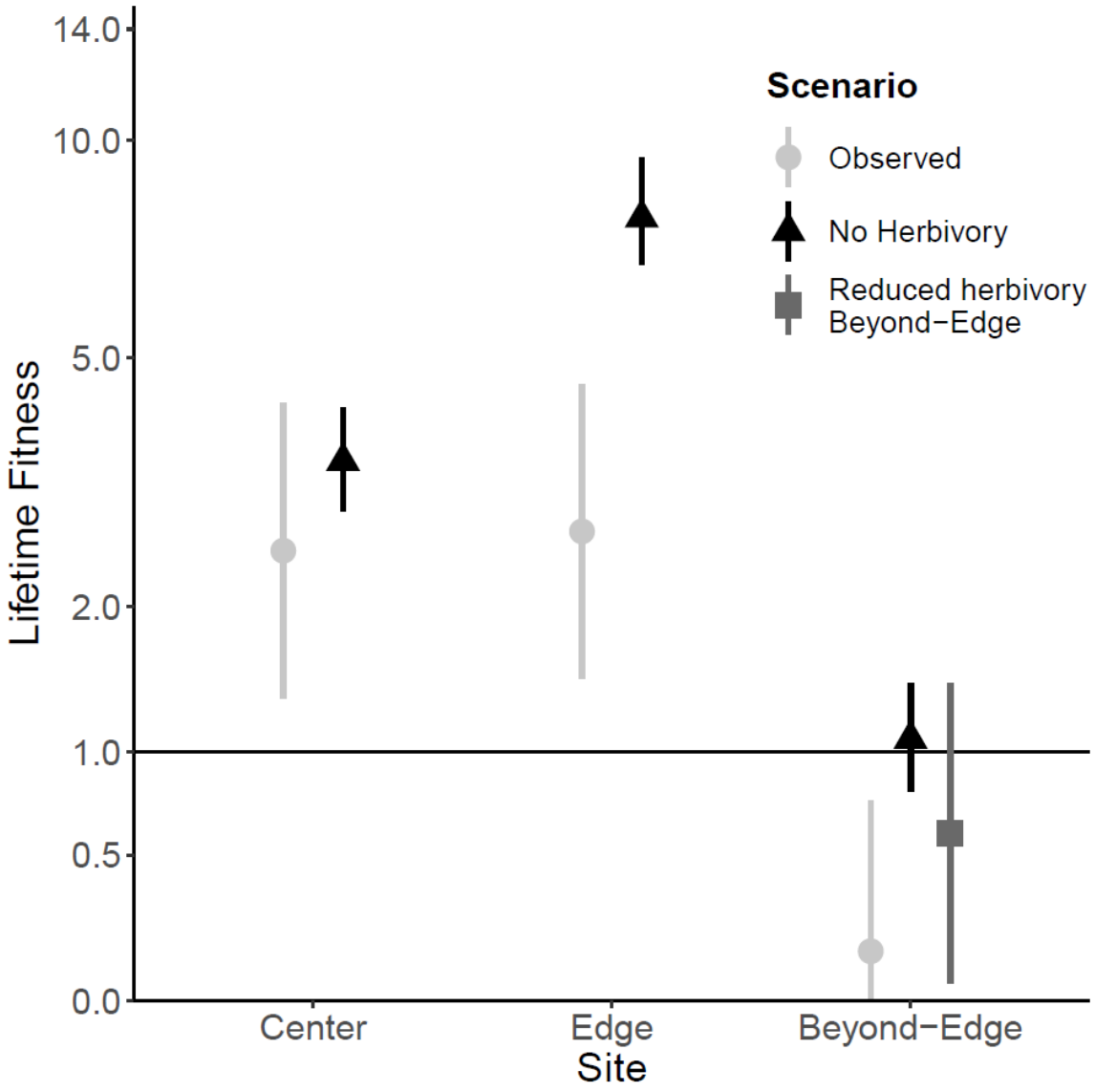

Figure 1.3. Lifetime fitness estimates (and 95% confidence intervals) for *xantiana* in the Wet Year with observed (i.e., unsimulated) values (light grey circles) and under two simulated scenarios: "No Herbivory at Any Site" (black triangles), where we predicted fitness values for all plants eaten during the field experiment as if they hadn't been eaten; and "Reduced herbivory Beyond-Edge" (dark grey square), where we simulated lowered herbivory rates outside *xantiana*'s range limit. Note Y axis is on log scale. N = 4,185 planting positions. Upper and lower confidence limits for simulation estimates are the 97.5% and 2.5% quantiles of the set of 100 estimated means.

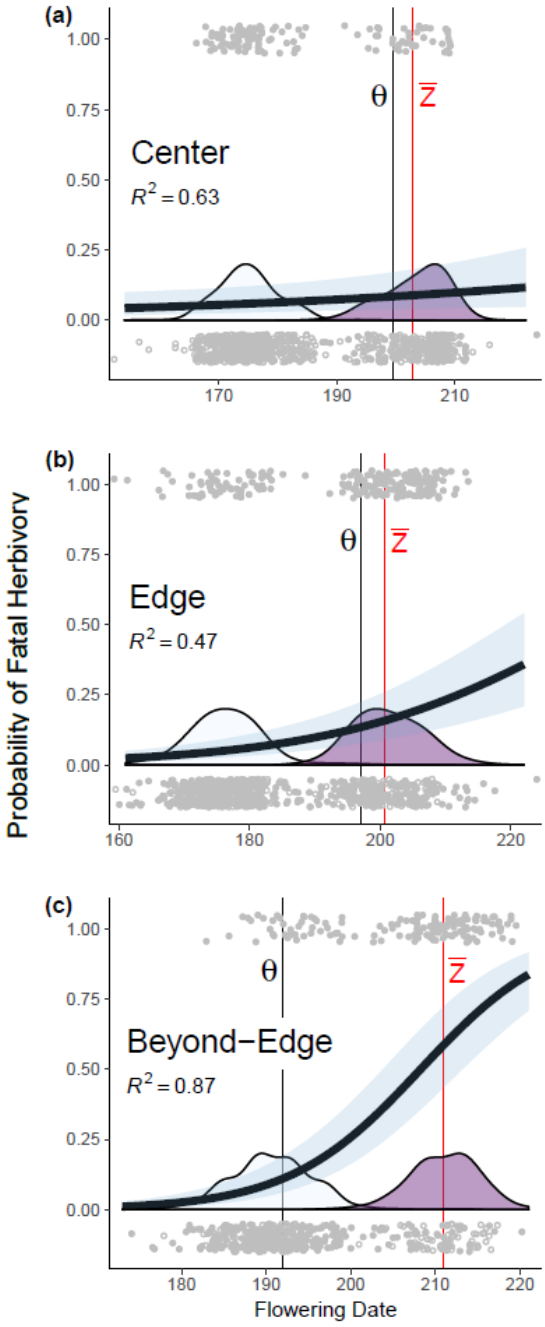

Figure 1.4. Conditional effects of phenology on probability of fatal herbivory (with 95% CI bands) as modeled by logistic regression, holding size and block constant, at Center (a), Edge (b), and Beyond-Edge (c) sites in the Wet Year. Kernel density estimates (smoothed histograms) indicate distribution of flowering date for each subspecies (light blue = *parviflora*; purple = *xantiana*). Jittered points are individual plants that either did or did not experience herbivory. Open points indicate plants that died due to factors other than herbivory. Optimal flowering date, where fitness was maximized, is marked by the black line labeled θ. The mean *xantiana* flowering date is marked by the red line labeled $\overline{Z}$. N = 8,488 planting positions.

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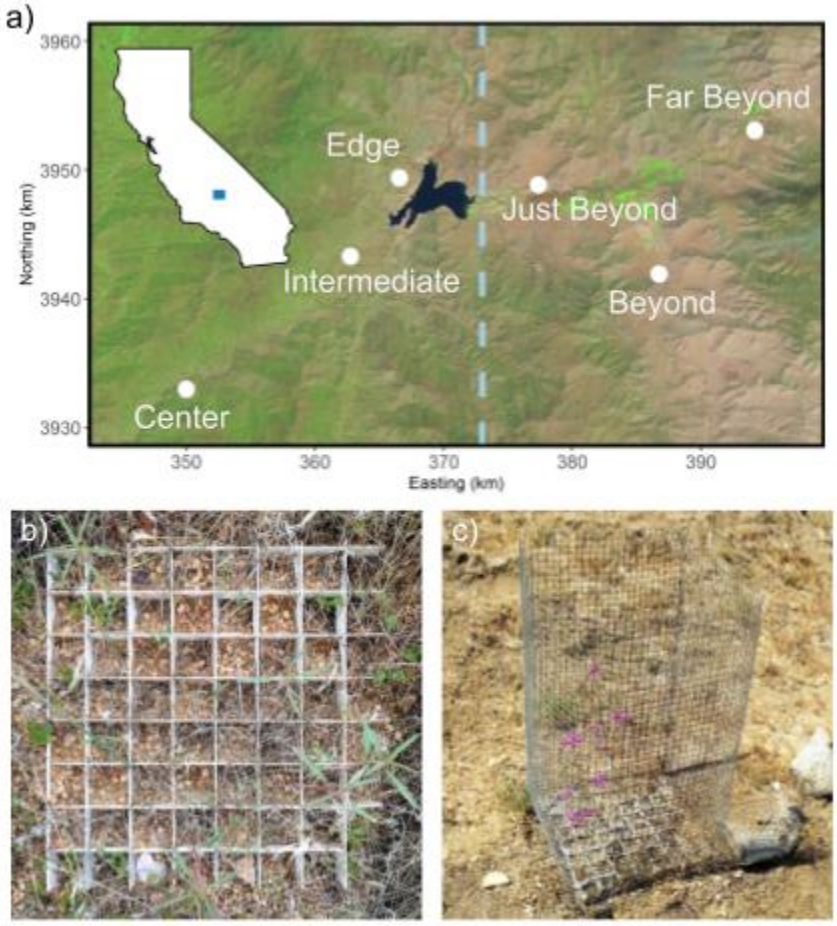

Figure 2.1. Overview of study area and implementation of the experiment. a) Study area in Southern California and the locations of transplant sites (circles). The dashed blue line marks *xantiana*'s eastern range limit. Background image is 19 April 2016 LANDSAT imagery of study area. Axes are UTM coordinates; Zone 11 S. b) Planting grid installed in the ground, with *xantiana* seedlings visible at top left and bottom right. c) Caged grid around flowering *xantiana* individuals.

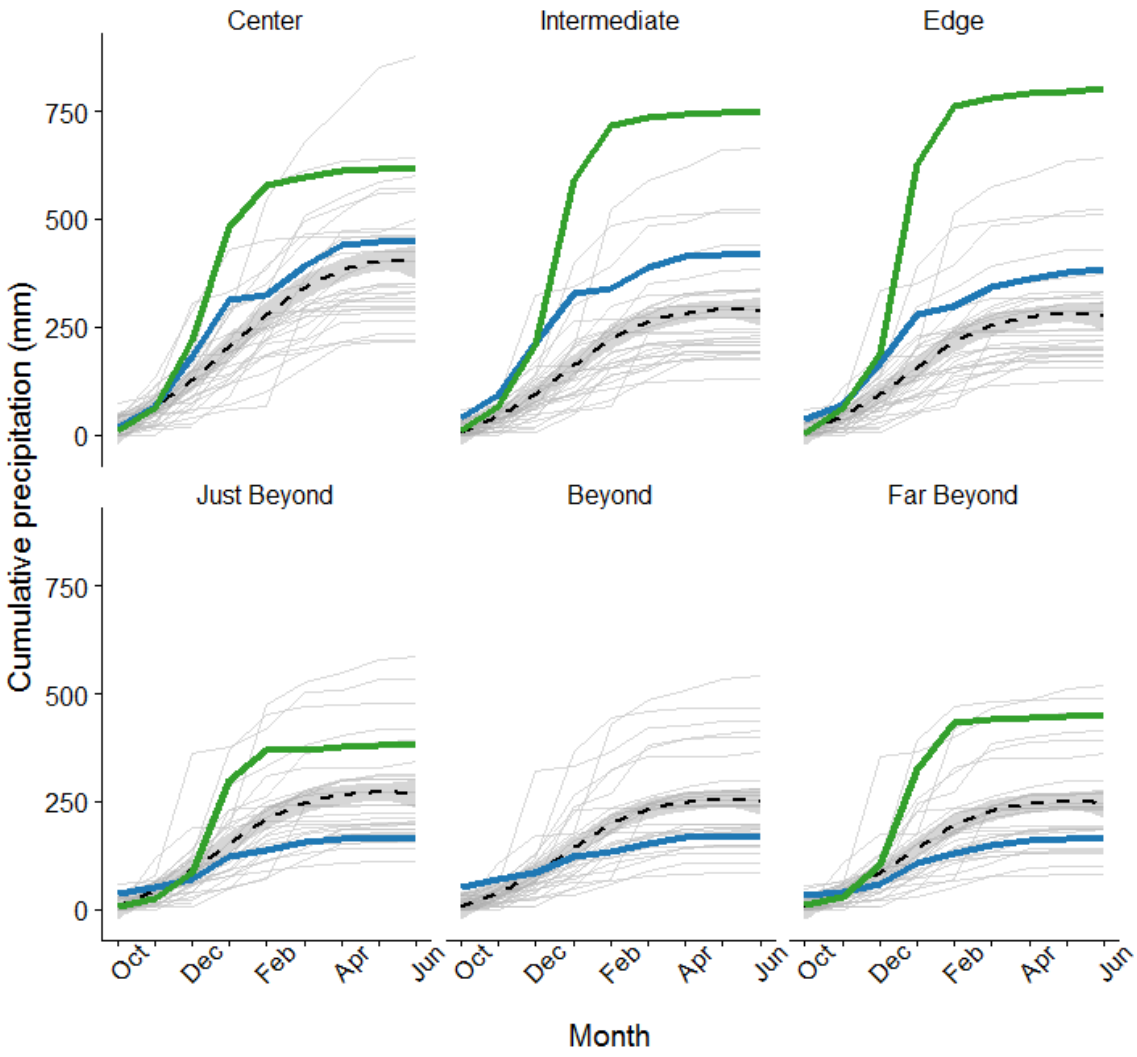

Figure 2.2. Cumulative precipitation across the growing season (October - June) within the study area. Shown are precipitation patterns during the transplant experiment (year 1: blue lines; year 2: green lines), using data from weather stations at or near the sites. We also plotted precipitation for the years 1990 - 2017 at each site location (thin grey lines), using interpolated estimates from PRISM, to help interpret study year precipitation patterns in the context of long term trends (dashed black line shows long term trend with 95% confidence band). Precipitation data for the Beyond site in year 2 was unavailable due to a wildfire destroying our weather station.

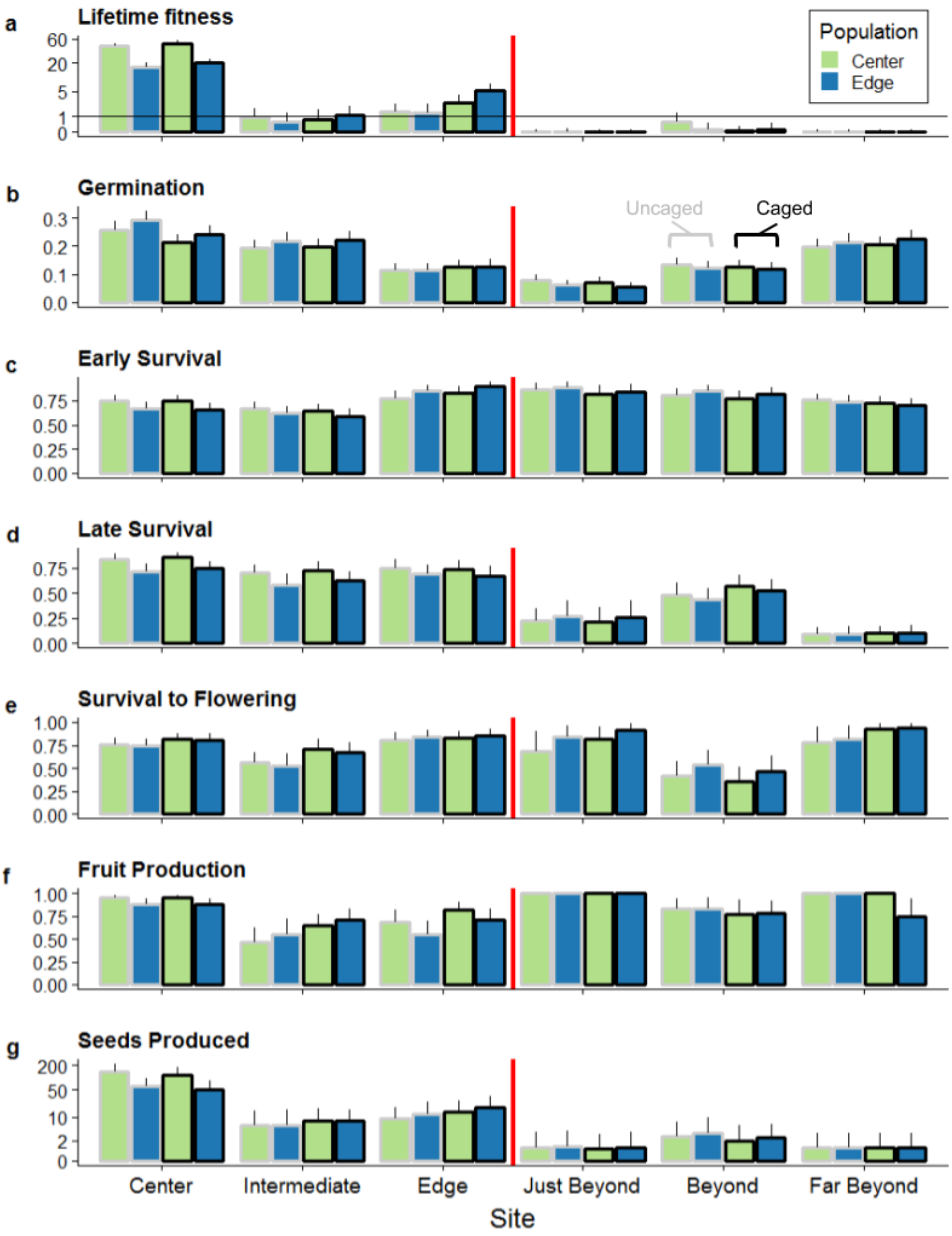

Figure 2.3. *Xantiana* mean lifetime fitness in year 1 estimated from the full *aster* model (panel a) and least squares means for components of lifetime fitness (panels b-g), by site, population, and caging treatment. Caged treatment is indicated by a black border around the bar. Significant differences in lifetime fitness between caging treatments are indicated with horizontal black bars; numbers above bars are deviance values from LRT test of the effect of caging at that site. The red line demarcates sites within the range (left), and outside the range (right). Lifetime fitness (panel a) and seeds (panel g) are on the log scale to aid visualization. All line ranges are 95% confidence intervals. Following germination, fitness component analyses are "conditional" — i.e., only those plants that had non-zero values for the preceding fitness component are analyzed. Thus, early survival shows the probability of a germinated seed surviving until March.

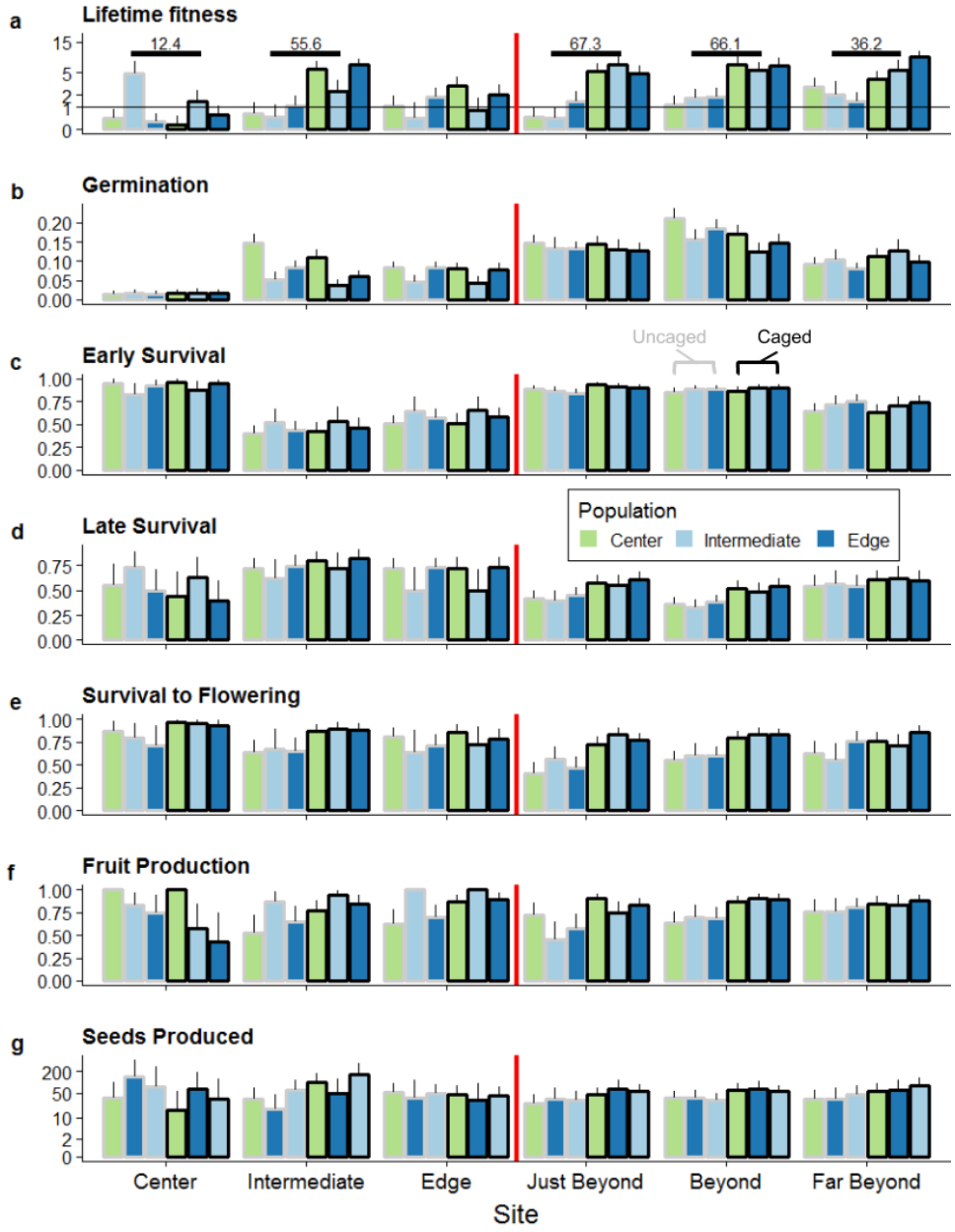

Figure 2.4. *Xantiana* mean lifetime fitness in year 2 estimated from the full *aster* model (panel a) and least squares means for components of lifetime fitness (panels b-g), by site, population, and caging treatment. Caged treatment is indicated by a black border around the bar. Significant differences in lifetime fitness between caging treatments are indicated with horizontal black bars; numbers above bars are deviance values from LRT test of the effect of caging at that site. The red line demarcates sites within the range (left), and outside the range (right). Lifetime fitness (panel a) and seeds (panel g) are on the log scale to aid visualization. All line ranges are 95% confidence intervals. Following germination, fitness component analyses are "conditional" — i.e., only those plants that had non-zero values for the preceding fitness component are analyzed. Thus, early survival shows the probability of a germinated seed surviving until March.

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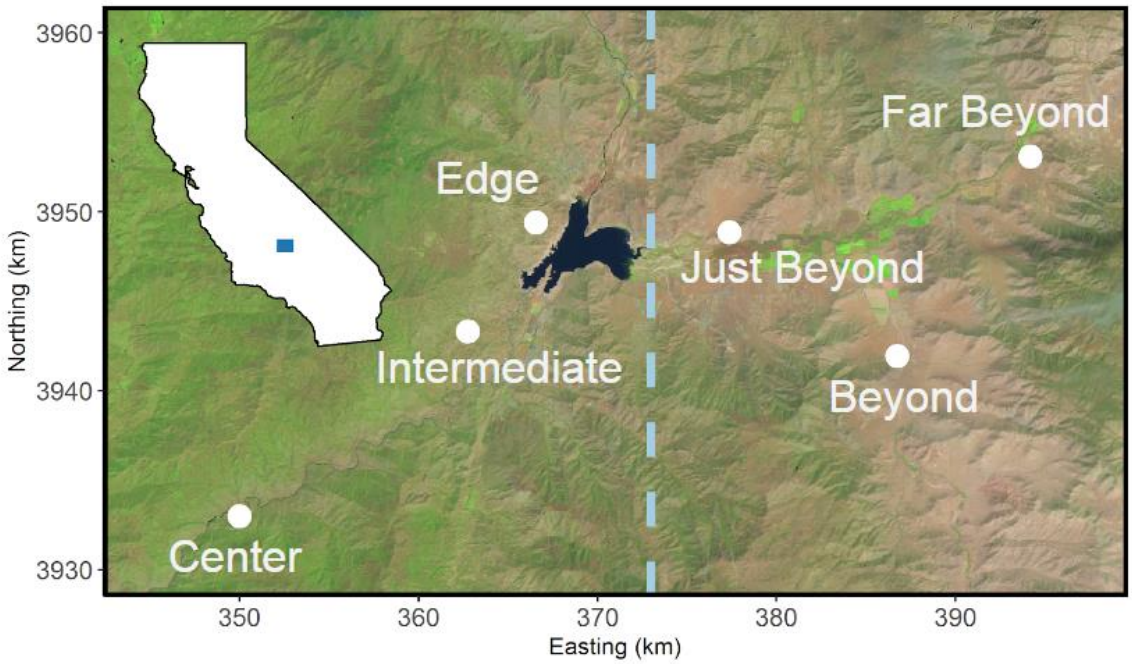

Figure 3.1. Overview of study area in Southern California and the locations of sites used in the greenhouse and field experiments. The dashed blue line marks *xantiana*'s eastern range limit. Background image is 19 April 2016 LANDSAT imagery of study area. Axes are UTM coordinates; Zone 11 S.

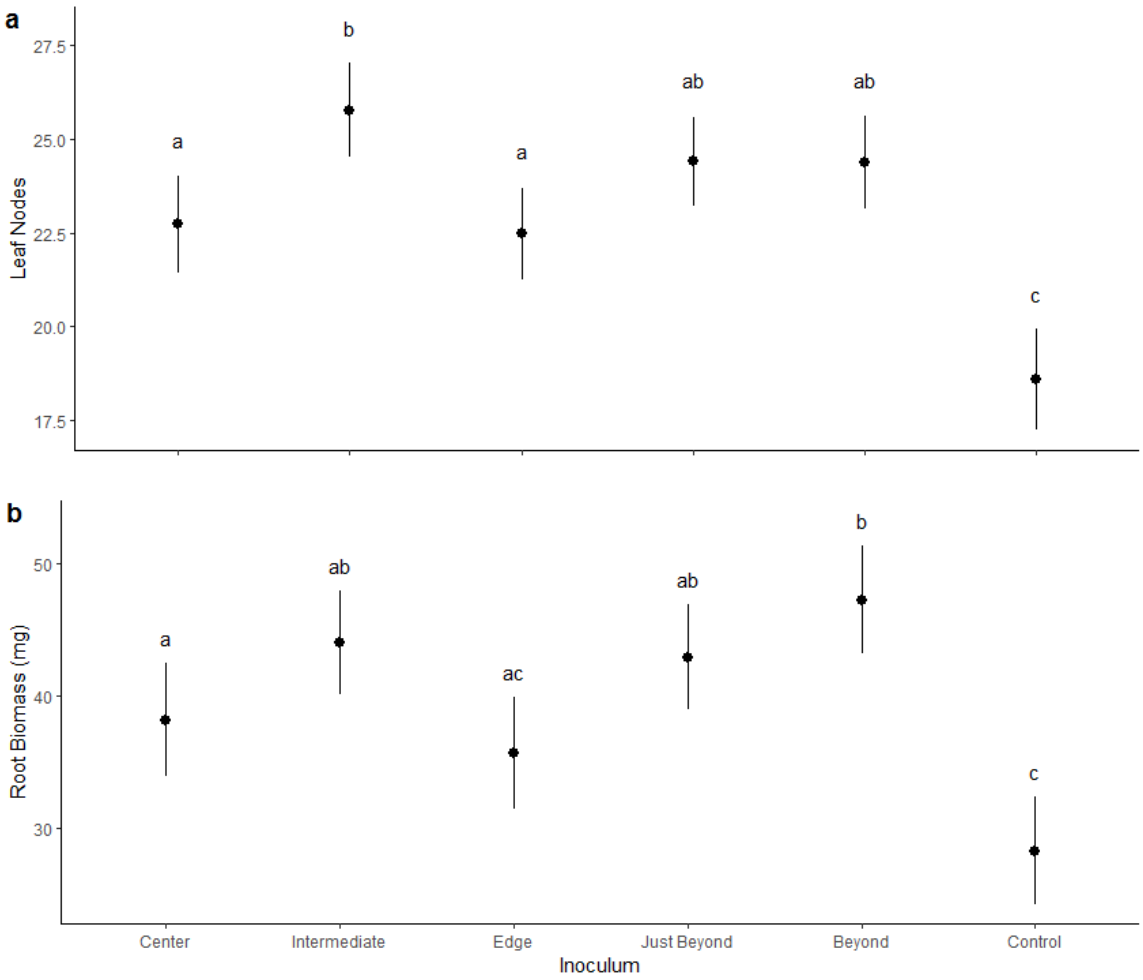

Figure 3.2. Effect of microbial inocula from within and beyond *xantiana*'s range on **a)** leaf node number and **b)** root biomass in the greenhouse. Estimates and 95% confidence intervals are estimated marginal means from linear models of each response on source population, inoculum, and their interaction, averaging over source populations and benches. Letters indicate Tukey groupings at $\alpha = 0.05$.

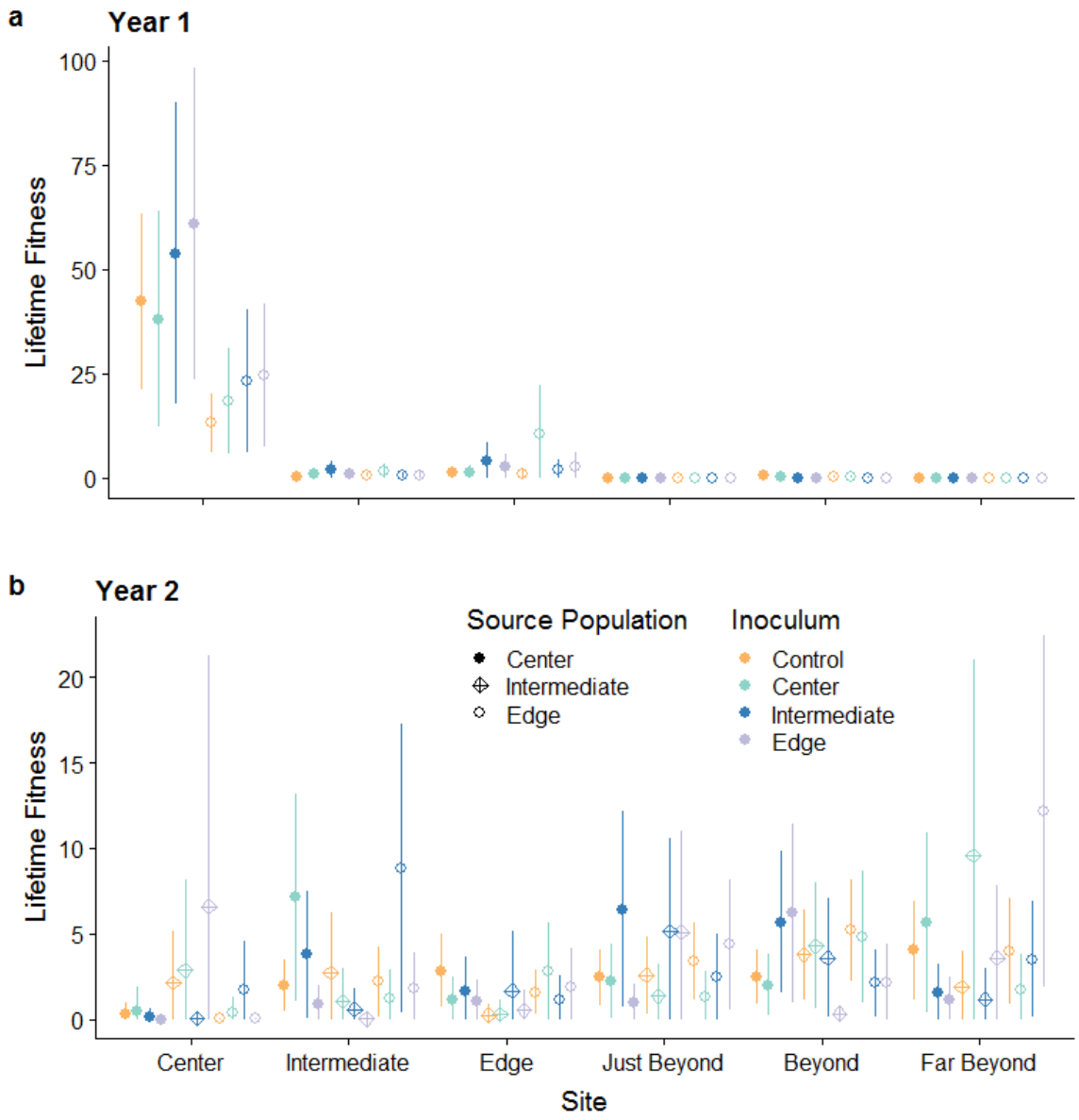

Fig. 3.3. Estimated mean lifetime fitness (± 95% CI) across sites, source populations, and inoculum treatments for the field experiment in years **a)** 1 and **b)** 2, as estimated from *aster* models.

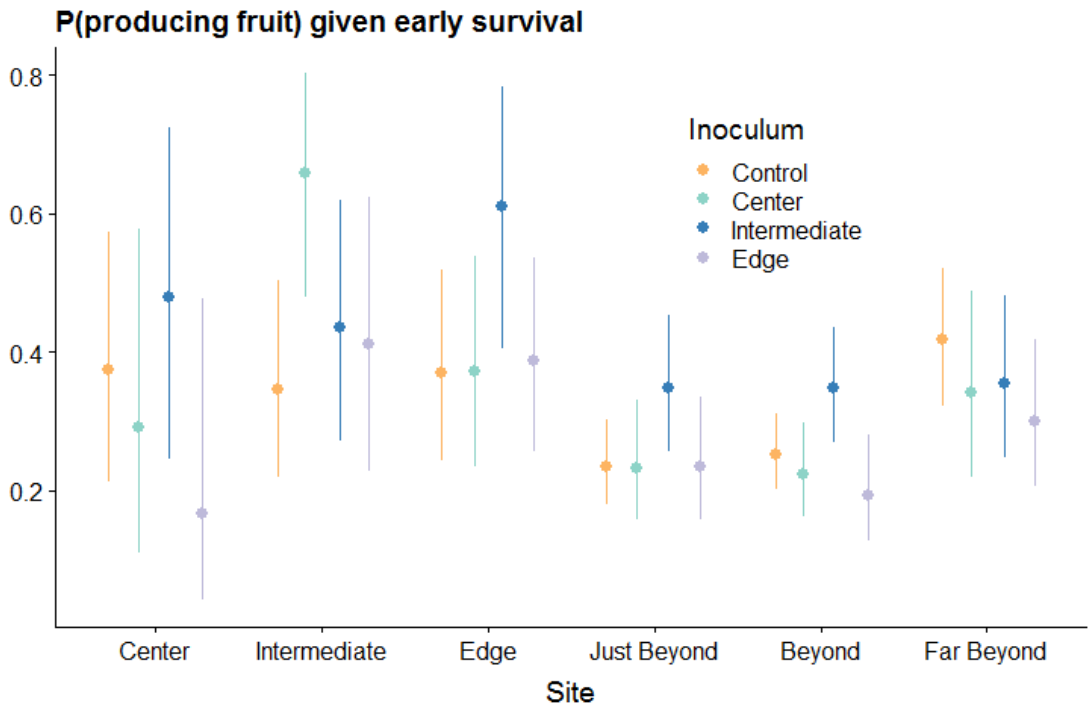

Figure 3.4. Probability of producing any fruits, given early survival, for each inoculum treatment in the field experiment. Estimates (± 95% CI) are estimated marginal means from the logistic regression of fruit production on site, source population, inoculum, and their interactions, averaging over source populations.

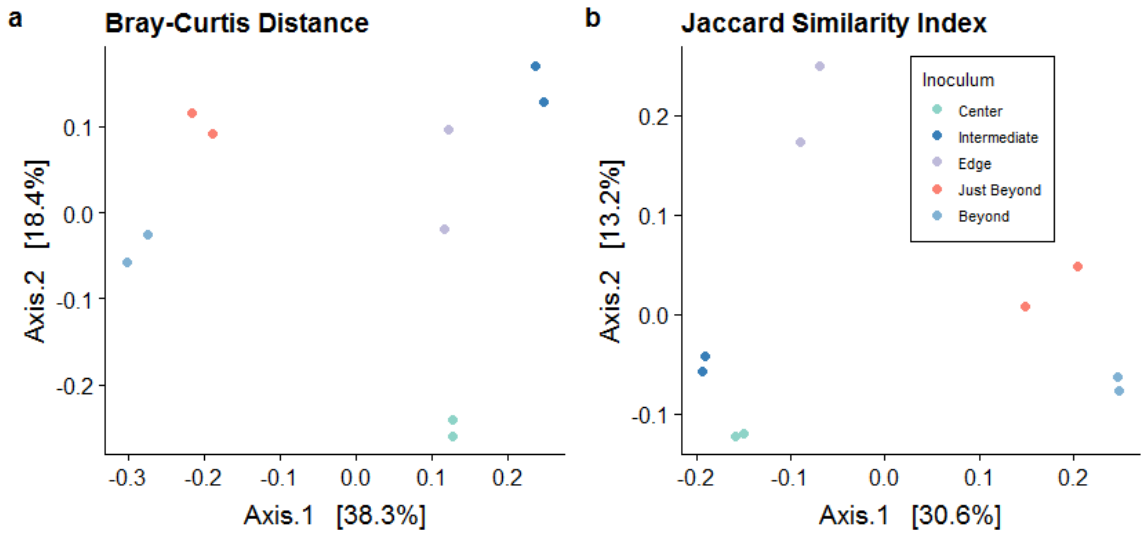

Figure 3.5. PCoA of **a)** Bray-Curtis distance and **b)** Jaccard similarity index matrices comparing community composition among inoculum sources from the greenhouse experiment.

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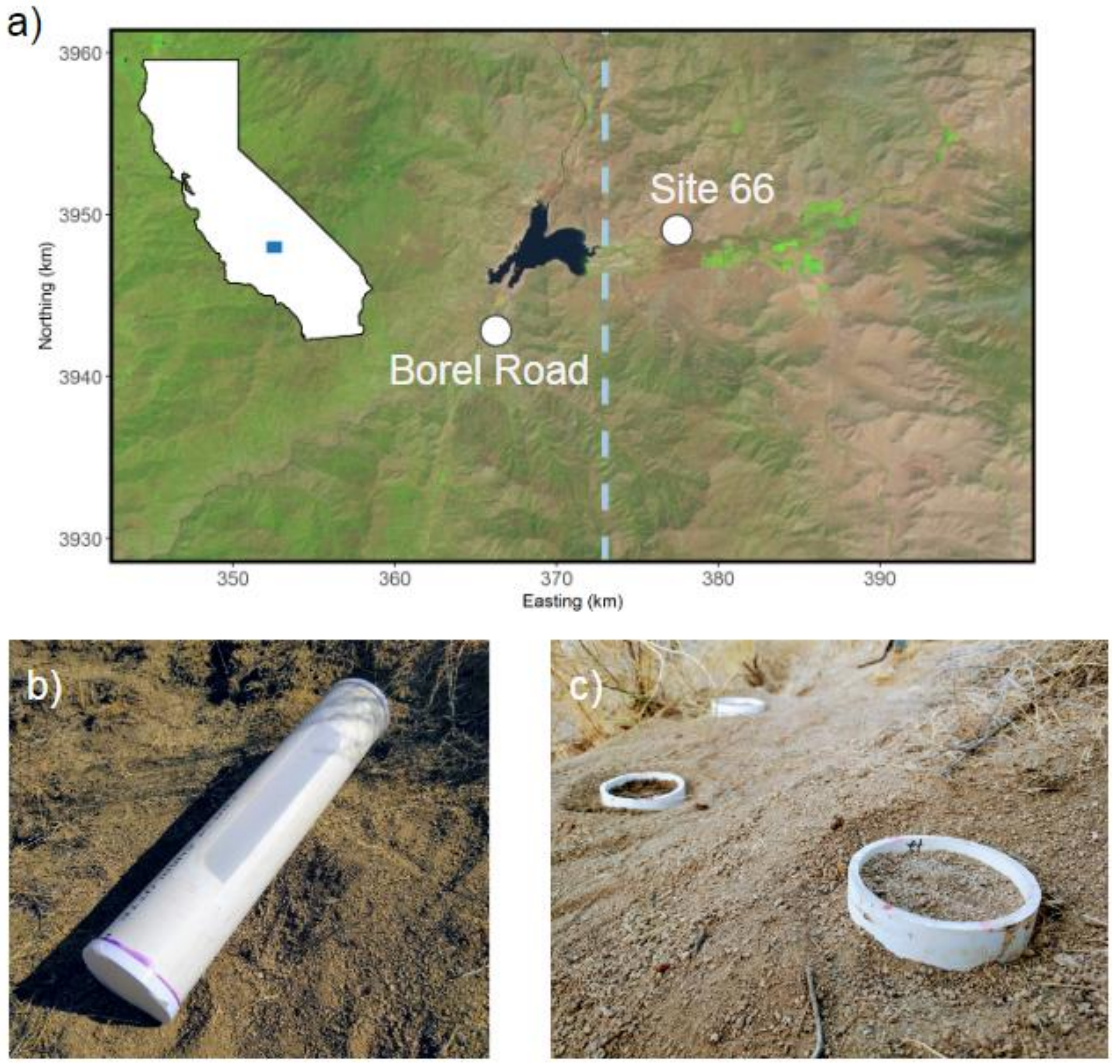

Figure 4.1. a) Overview of study area in Southern California and the locations of sites used in the field experiment; seeds were sourced from the Borel Road site and the experiment took place at Site 66, just outside *xantiana*'s eastern range limit, marked by the dashed blue line. Background image is 19 April 2016 LANDSAT imagery of study area. Axes are UTM coordinates; Zone 11 S. **b)** One of the mesocosms used to manipulate edaphic environments in the field, before installation in the ground. Note the mesh-covered openings on two sides (only one shown) and bottom. **c)** Mesocosms installed in the ground at Site 66 and filled with soil.

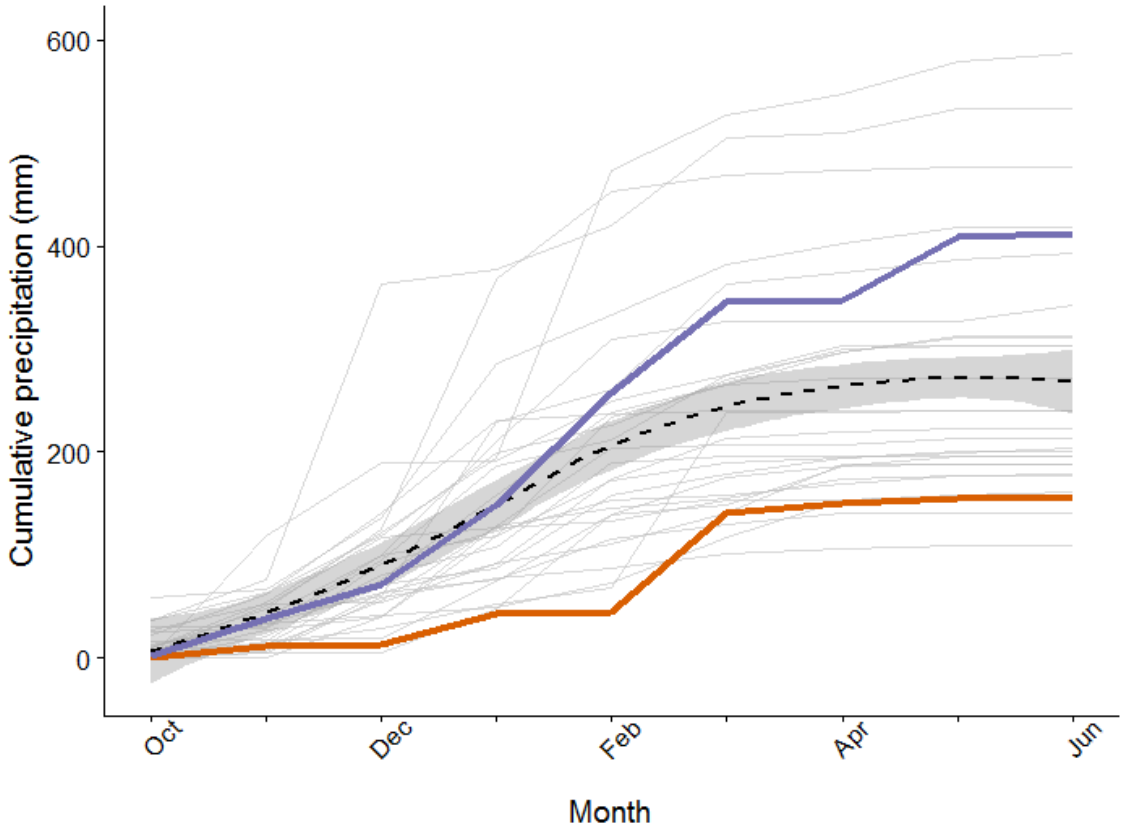

Figure 4.2. Growing season precipitation at the experimental site for years 1 (orange line) and 2 (purple line), and long term trends for years 1991 - 2019 (thin grey lines) as interpolated by PRISM. Dashed black line is long term average with 95% confidence band, as determined by loess smoothing.

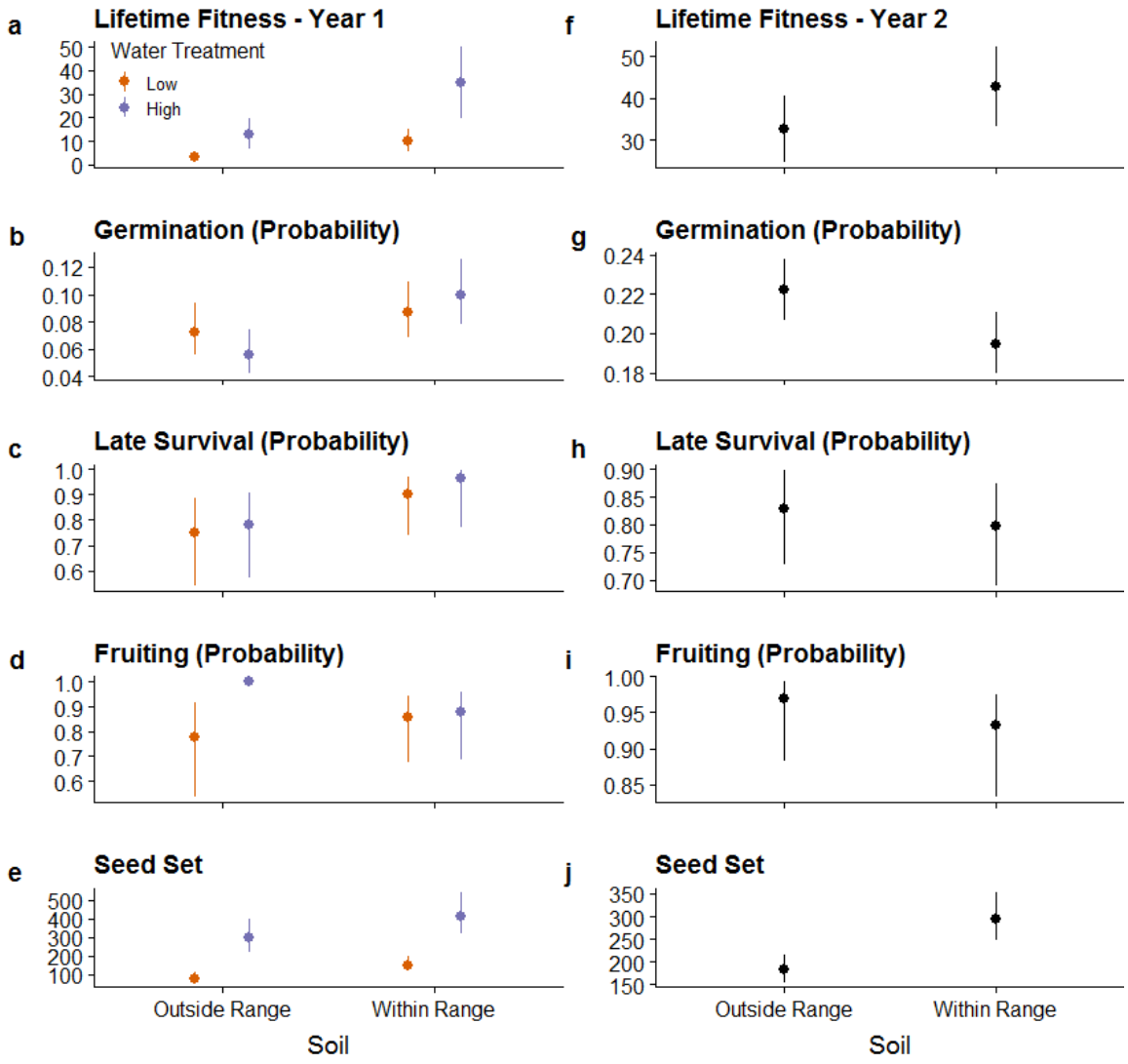

Fig. 4.3. Effects of soil and water treatments on **a)** lifetime fitness (seeds per planted seed) and **b - e)** conditional components of lifetime fitness in year 1 of the experiment, and effects of soil treatments on **f)** lifetime fitness and **g - j)** conditional components of lifetime fitness in year 2 of the experiment. Estimates of seeds produced per planted seed (lifetime fitness) were calculated as the product of germination probabilities and fitness estimates from an *aster* model including only those plants that germinated (see Methods). All error bars are 95% CI's.

Tables – Chapter 2

Table 2.1. Summary of results from *aster* model comparisons testing effects of site, population (Pop), caging treatment (Caged), and their interactions, on lifetime fitness in *xantiana*, in both years of the experiment.

	Year 1				Year 2			
Term	**Resid. df**	**Test df**	**Dev**	***P***	**Resid. df**	**Test df**	**Dev**	***P***
Full	29				42			
Planting Year					41	1	30.29	**<0.001**
Site x Caged x Pop	24	5	1.52	0.91	32	10	30.63	**< 0.001**
First Order Interactions	24				32			
Caged x Pop	23	1	0.98	0.32	30	2	0.84	0.66
Site x Caged	18	5	5.81	0.32	25	5	80.71	**< 0.001**
Site x Pop	13	5	29.25	**< 0.001**	20	10	104.28	**< 0.001**
Main effects only	13				15			
Caged	12	1	7.32	**0.007**	14	1	159.57	**< 0.001**
Pop	12	1	93.34	**< 0.001**	13	2	5.07	0.08
Site	8	5	1366.5	**< 0.001**	8	5	80.02	**< 0.001**

Table 2.2. Summary of Type II Analysis of Deviance for logistic regressions (Germination through Fruit Production) and linear regression (Seeds Produced) testing effects of site, population, caging treatment, and their interactions, on sequential components of *xantiana* lifetime fitness in year one.

		Year 1					
Term	**df**	**Germination χ^2**	**Early Survival χ^2**	**Late Survival χ^2**	**Survival to Flowering χ^2**	**Fruit Production χ^2**	**Seeds Produced (F)**
Site	5	463.45***	62.87***	455.70***	66.30***	72.61***	1092.00***
Population	1	2.16	0.96	7.17**	0.20	2.65	8.85
Caged	1	0.76	0.62	1.14	3.93*	3.48 ^	0.00
Site x Population	5	7.77	8.28	5.62	2.81	7.76	25.45
Site x Caged	5	8.54	2.50	1.27	4.33	6.05	8.38

^ $P < 0.1$
* $P < 0.05$
** $P < 0.001$
*** $P < 0.0001$

Table 2.3. Summary of Type II Analysis of Deviance for logistic regressions (Germination through Fruit Production) and linear regression (Seeds Produced) testing effects of site, population, caging treatment, and their interactions, on sequential components of *xantiana* lifetime fitness in year two.

		Year 2					
Term	**df**	**Germination** χ^2	**Early Survival** χ^2	**Late Survival** χ^2	**Survival to Flowering** χ^2	**Fruit Production** χ^2	**Seeds produced (F)**
Site	5	1293.67***	371.73***	83.19***	22.36***	4.50	1.15
Population	2	41.14**	5.09^	1.84	1.10	0.09	0.65
Caged	1	5.90*	1.20	24.33***	55.46***	30.28***	13.04***
Plant Year	1	353.84***					
Site x Population	10	56.56***	10.21	8.57	9.65	19.23*	1.10
Site x Caged	5	22.93***	3.21	8.09	5.37	9.93^	2.23
Site x Plant Year	5	391.32***					

^ P < 0.1
* P < 0.05
** P < 0.001
*** P < 0.0001

Tables – Chapter 3

Table 3.1. Summary of Type II ANOVAs testing effects of population, inoculum, and their interaction, and bench position, on *xantiana* phenotypic traits in the greenhouse. Values are *F* ratios, with asterisks indicating significance of term in Type II tests. Bolded values remain significant after adjusting for multiple tests with the Holm method. Root mass was measured on ca. 70% of the experimental plants, hence the lower residual degrees of freedom.

		Traits		
		Time to Flowering	**Root Mass**	**Nodes**
Term	**df**	Res. df = 427	Res. df = 301	Res. df = 433
Population	2	**84.7 *****	**31.1*****	**27.8*****
Inoculum	5	0.9	**11.3*****	**14.0*****
Bench	3	**33.5 *****	0.6	**9.7*****
Population x Inoculum	10	2.2*	0.7	0.7

Table 3.2. Summary of results from *aster* LRT model comparisons testing effects of site, population, inoculum treatment, and their interactions, on lifetime fitness in *xantiana*, in both years of the experiment.

	Year 1				Year 2			
Term	**Model Parameters**	**Test df**	**Dev**	***P***	**Model Parameters**	**Test df**	**Dev**	***P***
Full	51				76			
Site x Pop x Inoculum	36	15	10.1	0.81	46	30	90.0	< **0.001**
First Order Interactions	36				46			
Inoculum x Pop	33	3	1.4	0.69	40	6	7.6	0.27
Site x Inoculum	21	15	28.8	**0.02**	31	15	23.6	0.07
Site x Pop	31	5	5.3	0.38	36	10	63.2	<**0.001**
Main effects only	13				15			
Plant Year					14	1	13.4	<**0.001**
Inoculum	10	3	2.8	0.42	12	3	1.0	0.79
Pop	12	1	13.3	<**0.001**	13	2	2.0	0.37
Site	8	5	617	<**0.001**	10	5	35.1	<**0.001**

Table 3.3. Summary of Type II Analysis of Deviance for logistic regressions (Germination through Survival to Fruiting) and negative binomial regression (Seed Set) testing effects of site, population (Pop), inoculum (Inoc), and their interactions, on sequential components of *xantiana* lifetime fitness in years 1 and 2. Values are likelihood ratio χ^2 statistics. Bolded values remain significant after Holm adjustment.

	Lifetime fitness components									
		Year 1					**Year 2**			
		Germ-ination	**Early Survival**	**Survival to Fruiting**	**Seed Set**		**Germ-ination**	**Early Survival**	**Survival to Fruiting**	**Seed Set**
Term	**df**	Res. df = 7,109	Res. df = 1,795	Res. df = 1,264	Res. df = 270	**df**	Res. df = 14,218	Res. df = 2,340	Res. df = 1,694	Res. df = 477
Site	5	**509.6*** **	**47.9*** **	**395.9*** **	**168.5*** **	5	**1156.8*** **	**347.0*** **	**39.4*** **	**28.1*** **
Pop	1	2.5	0.7	**6.6***	**8.1** **	2	**35.3*** **	5.0	1.5	2.7
Inoc	3	6.2	5.3	0.4	8.8*	3	2.1	7.7	**12.3****	1.7
Plant year						1	**319.6*** **			
Site x Pop	5	8.0	7.3	8.0	**13.9** **	10	**60.1*** **	9.5	6.7	16.9
Site x Inoc	15	23.7	19.6	17.5	14.4	15	27.3*	19.3	18.2	**40.9*** **
Pop x Inoc	3	1.3	2.4	0.6	7.4	6	4.5	4.1	3.0	8.7
Site x Plant year						5	**369.0*** **			
Site x Pop x Inoc	15	25.2*	10.6	8.8	4.5	30	43.9*	46.3*	29.5	**69.5*** **

Table 3.4. Summary of ASV richness and diversity for all inoculum sources used in the greenhouse experiment, after pooling across samples within inocula and for individual samples.

	Richness (Number of Observed ASVs)			**Shannon Diversity Index**		
Inoculum source	***Full Community***	***Bacteria***	***Fungi***	***Full Community***	***Bacteria***	***Fungi***
Center	3267	2793	474	6.9	7.1	4.9
Sample 1	2594	2256	338	6.7	7.0	4.8
Sample 2	2780	2409	371	6.8	7.0	4.8
Intermediate	3223	2950	273	7.3	7.3	4.7
Sample 1	2895	2659	236	7.2	7.2	4.6
Sample 2	2461	2305	156	7.2	7.1	4.1
Edge	3360	2931	429	7.2	7.2	5.0
Sample 1	2531	2136	215	7.0	7.0	4.5
Sample 2	3021	2646	375	7.1	7.2	4.9
Just Beyond	2922	2641	281	6.7	6.9	4.1
Sample 1	2520	2332	188	6.6	6.9	3.8
Sample 2	2171	1978	193	6.5	6.8	4.0
Beyond	2920	2581	339	6.7	6.7	4.4
Sample 1	2199	2020	179	6.7	6.6	4.0
Sample 2	2508	2200	308	6.6	6.7	4.4

Tables – Chapter 4

Table 4.1. Summary of results from LRT *aster* model comparisons testing effects of soil and water treatments, and their interaction, on lifetime fitness in *xantiana* in year 1, and the effect of soil treatment on lifetime fitness in year 2.

	Year 1			Year 2		
Term	**Resid. df**	**Test df**	**Dev**	**Resid. df**	**Test df**	**Dev**
First Order Interaction	7					
Soil × *Water*	6	1	3.0†			
Main effects only	8			5		
Soil	5	3	**10.1****	4	1	**5.3***
Water	7	1	**23.3*****			

† = $P < 0.1$

The germination node of these aster models is modeled as a bernoulli variable (i.e., germinants were or were not present). Because we sowed multiple seeds into each mesocosm, for Fig. 3, estimates of seeds produced per planted seed were calculated as the product of germination probabilities and fitness estimates from an aster model including only those plants that germinated (see Methods).

Table 4.2. Summary of Type II Analysis of Deviance for logistic regressions (Germination through Survival to Fruiting) and negative binomial regression (Seed Set) testing effects of soil treatment, water treatment, and their interaction, on sequential components of *xantiana* lifetime fitness in year 1, and the effect of soil treatment on these fitness components in year 2. Values are likelihood ratio χ^2 statistics. Bolded values remain significant at $P < 0.05$ after Holm adjustment.

	Conditional lifetime fitness components									
	Year 1					**Year 2**				
Term	**df**	**Germination**	**Late Survival**	**Fruit Production**	**Seed Set**	**df**	**Germination**	**Late Survival**	**Fruit Production**	**Seed Set**
		Res. df = 132	Res. df = 100	Res. df = 85	Res. df = 74		Res. df = 154	Res. df = 148	Res. df = 120	Res. df = 114
Soil	1	**8.0****	**5.8***	0.1	**9.8****	1	**5.9***	0.2	0.9	**14.6****
Water	1	0.1	0.5	2.3	**56.1****					
Soil × *Water*	1	2.3	0.4	3.8†	1.1					

† = $P < 0.1$

Bibliography

Afkhami, M. E., P. J. McIntyre, and S. Y. Strauss. 2014. Mutualist-mediated effects on species' range limits across large geographic scales. Ecol. Lett. 17:1265–1273. Wiley Online Library.

Ahanger, M. A., N. Morad-Talab, E. F. Abd-Allah, P. Ahmad, and R. Hajiboland. 2016. Plant growth under drought stress: Significance of mineral nutrients. Pp. 649–668 *in* P. Ahmad, ed. Water Stress and Crop Plants. John Wiley & Sons, Ltd, Chichester, UK.

Alexander, J. M., L. Chalmandrier, J. Lenoir, T. I. Burgess, F. Essl, S. Haider, C. Kueffer, K. McDougall, A. Milbau, M. A. Nuñez, A. Pauchard, W. Rabitsch, L. J. Rew, N. J. Sanders, and L. Pellissier. 2018. Lags in the response of mountain plant communities to climate change. Glob. Chang. Biol. 24:563–579.

Alexander, J. M., J. M. Diez, and J. M. Levine. 2015. Novel competitors shape species' responses to climate change. Nature 525:515–518. Nature Research.

Anderson, J. P., C. A. Gleason, R. C. Foley, P. H. Thrall, J. B. Burdon, and K. B. Singh. 2010. Plants versus pathogens: an evolutionary arms race. Funct. Plant Biol. 37:499–512. CSIRO.

Anderson, J. T., V. M. Eckhart, and M. A. Geber. 2015. Experimental studies of adaptation in Clarkia xantiana. III. Phenotypic selection across a subspecies border. Evolution 69:2249–2261.

Angert, A. L. 2006. Growth and leaf physiology of monkeyflowers with different altitude ranges. Oecologia 148:183–194.

Angert, A. L., H. D. Bradshaw Jr, and D. W. Schemske. 2008. Using experimental evolution to investigate geographic range limits in monkeyflowers. Evolution 62:2660–2675.

Angert, A. L., and D. W. Schemske. 2005. The evolution of species' distributions: reciprocal transplants across the elevation ranges of Mimulus cardinalis and M. Lewisii. Evolution. Wiley Online Library.

Antonovics, J. 1987. The Evolutionary Dys-Synthesis: Which Bottles for Which Wine? Am. Nat. 129:321–331. [University of Chicago Press, American Society of Naturalists].

Antonovics, J. 1976. The Nature of Limits to Natural Selection. Ann. Mo. Bot. Gard. 63:224–247. Missouri Botanical Garden Press.

Aragón, P., and D. Sánchez-Fernández. 2013. Can we disentangle predator--prey interactions from species distributions at a macro-scale? A case study with a raptor species. Oikos 122:64–72. Wiley Online Library.

Araújo, M. B., and M. Luoto. 2007. The importance of biotic interactions for modelling species distributions under climate change. Glob. Ecol. Biogeogr. 16:743–753. Blackwell Publishing Ltd.

Arias-Del Razo, I., L. Hernández, J. W. Laundré, and L. Velasco-Vázquez. 2012. The landscape of fear: habitat use by a predator (Canis latrans) and its main prey (Lepus californicus and Sylvilagus audubonii). Can. J. Zool. 90:683–693. NRC Research Press.

Aronson, J., J. Kigel, A. Shmida, and J. Klein. 1992. Adaptive phenology of desert and Mediterranean populations of annual plants grown with and without water stress. Oecologia 89:17–26.

Augé, R. M. 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11:3–42. Springer-Verlag.

Baer, K. C., and J. L. Maron. 2018. Pre-dispersal seed predation and pollen limitation constrain population growth across the geographic distribution of Astragalus utahensis. J. Ecol. 106:1646–1659.

Beauregard, F., and S. de Blois. 2014. Beyond a climate-centric view of plant distribution: edaphic variables add value to distribution models. PLoS One 9:e92642. journals.plos.org.

Bennett, A. E., J. D. Bever, and M. Deane Bowers. 2009. Arbuscular mycorrhizal fungal species suppress inducible plant responses and alter defensive strategies following herbivory. Oecologia 160:771–779.

Berns, A. E., H. Philipp, H.-D. Narres, P. Burauel, H. Vereecken, and W. Tappe. 2008. Effect of gamma-sterilization and autoclaving on soil organic matter structure as studied by solid state NMR, UV and fluorescence spectroscopy. Eur. J. Soil Sci. 59:540–550.

Bertrand, R., V. Perez, and J.-C. Gégout. 2012. Disregarding the edaphic dimension in species distribution models leads to the omission of crucial spatial information under climate change: the case of Q uercus pubescens in F rance. Glob. Chang. Biol. 18:2648–2660. Wiley Online Library.

Bever, J. D., S. A. Mangan, and H. M. Alexander. 2015. Maintenance of Plant Species Diversity by Pathogens. Annu. Rev. Ecol. Evol. Syst. 46:305–325. Annual Reviews.

Biere, A., and K. J. F. Verhoeven. 2008. Local adaptation and the consequences of being dislocated from coevolved enemies.

Brady, S., D. I. Bolnick, R. D. H. Barrett, L. J. Chapman, E. Crispo, A. M. Derry, C. G. Eckert, D. J. Fraser, G. F. Fussmann, A. Gonzalez, F. Guichard, T. Lamy, J. E. Lane, A. G. McAdam, A. E. M. Newman, A. Paccard, B. Robertson, G. Rolshausen, P. M. Schulte, A. M. Simons, M. Vellend, and A. P. Hendry. 2019. Understanding maladaptation by uniting ecological and evolutionary perspectives. Am. Nat., doi: 10.1086/705020. The University of Chicago Press.

Bridle, J. R., M. Kawata, and R. K. Butlin. 2019. Local adaptation stops where ecological gradients steepen or are interrupted. Evol. Appl., doi: 10.1111/eva.12789.

Bridle, J. R., and T. H. Vines. 2007. Limits to evolution at range margins: when and why does adaptation fail? Trends Ecol. Evol. 22:140–147. Elsevier.

Briers, R. A. 2003. Range limits and parasite prevalence in a freshwater snail. Proc. Biol. Sci. 270 Suppl 2:S178–80.

Briscoe Runquist, R. D., E. Chu, J. L. Iverson, J. C. Kopp, and D. A. Moeller. 2014. Rapid evolution of reproductive isolation between incipient outcrossing and selfing Clarkia species. Evolution 68:2885–2900. Wiley Online Library.

Brown, C. D., and M. Vellend. 2014. Non-climatic constraints on upper elevational plant range expansion under climate change. Proceedings of the Royal Society B: Biological Sciences 281:20141779. Royal Society.

Bruelheide, H., and U. Scheidel. 1999. Slug herbivory as a limiting factor for the geographical range of Arnica montana. J. Ecol. 87:839–848. Blackwell Science Ltd.
Buehler, R. J. 1957. Confidence Intervals for the Product of Two Binomial Parameters. J. Am. Stat. Assoc. 52:482–493. [American Statistical Association, Taylor & Francis, Ltd.].
Bullock, J. M., R. J. Edwards, P. D. Carey, and R. J. Rose. 2000. Geographical separation of two Ulex species at three spatial scales: does competition limit species' ranges? Ecography 23:257–271. Blackwell Publishing Ltd.
Buri, A., C. Cianfrani, E. Pinto-Figueroa, E. Yashiro, J. E. Spangenberg, T. Adatte, E. Verrecchia, A. Guisan, and J.-N. Pradervand. 2017. Soil factors improve predictions of plant species distribution in a mountain environment. Progress in Physical Geography: Earth and Environment 41:703–722. SAGE Publications Ltd.
Callahan, B. J., P. J. McMurdie, and S. P. Holmes. 2017. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME J. 11:2639–2643.
Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods 13:581–583.
Caporaso, J. G., C. L. Lauber, W. A. Walters, D. Berg-Lyons, C. A. Lozupone, P. J. Turnbaugh, N. Fierer, and R. Knight. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc. Natl. Acad. Sci. U. S. A. 108 Suppl 1:4516–4522.
Case, T. J., R. D. Holt, M. A. McPeek, and T. H. Keitt. 2005. The community context of species' borders: ecological and evolutionary perspectives. Oikos. Wiley Online Library.
Case, T. J., and M. L. Taper. 2000. Interspecific Competition, Environmental Gradients, Gene Flow, and the Coevolution of Species' Borders. Am. Nat. 155:583–605.
Chardon, N. I., W. K. Cornwell, L. E. Flint, A. L. Flint, and D. D. Ackerly. 2015. Topographic, latitudinal and climatic distribution of Pinus coulteri: geographic range limits are not at the edge of the climate envelope. Ecography 38:590–601. Blackwell Publishing Ltd.
Chen, I.-C., J. K. Hill, R. Ohlemüller, D. B. Roy, and C. D. Thomas. 2011. Rapid range shifts of species associated with high levels of climate warming. Science 333:1024–1026.
Colautti, R. I., C. G. Eckert, and S. C. H. Barrett. 2010. Evolutionary constraints on adaptive evolution during range expansion in an invasive plant. Proc. Biol. Sci. 277:1799–1806.
Cregger, M. A., C. W. Schadt, N. G. McDowell, W. T. Pockman, and A. T. Classen. 2012. Response of the soil microbial community to changes in precipitation in a semiarid ecosystem. Appl. Environ. Microbiol. 78:8587–8594.
Cruz-Martínez, K., K. B. Suttle, E. L. Brodie, M. E. Power, G. L. Andersen, and J. F. Banfield. 2009. Despite strong seasonal responses, soil microbial consortia are more resilient to long-term changes in rainfall than overlying grassland. ISME J. 3:738–744.

Dawson, W., and M. Schrama. 2016. Identifying the role of soil microbes in plant invasions. J. Ecol. 104:1211–1218. Wiley Online Library.
deRivera, C. E., G. M. Ruiz, A. H. Hines, and P. Jivoff. 2005. Biotic resistance to invasion: native predator limits abundance and distribution of an introduced crab. Ecology 86:3364–3376. Ecological Society of America.
Diekmann, M., J. Michaelis, and A. Pannek. 2015. Know your limits – The need for better data on species responses to soil variables. Basic Appl. Ecol. 16:563–572.
Dubuis, A., S. Giovanettina, L. Pellissier, J. Pottier, P. Vittoz, and A. Guisan. 2013. Improving the prediction of plant species distribution and community composition by adding edaphic to topo-climatic variables. J. Veg. Sci. 24:593–606.
Duputié, A., F. Massol, I. Chuine, M. Kirkpatrick, and O. Ronce. 2012. How do genetic correlations affect species range shifts in a changing environment? Ecol. Lett. 15:251–259.
Eckhart, V. M., and M. A. Geber. 1999. Character variation and geographic range in Clarkia xantiana (Onagraceae): breeding system and phenology distinguish two common subspecies. Madrono.
Eckhart, V. M., M. A. Geber, and C. M. McGuire. 2004. Experimental studies of adaptation in Clarkia xantiana. I. Sources of trait variation across a subspecies border. Evolution 58:59–70.
Eckhart, V. M., M. A. Geber, W. F. Morris, E. S. Fabio, P. Tiffin, and D. A. Moeller. 2011. The geography of demography: long-term demographic studies and species distribution models reveal a species border limited by adaptation. Am. Nat. 178 Suppl 1:S26–43.
Eckhart, V. M., I. Singh, A. m. Louthan, A. j. Keledjian, A. Chu, D. a. Moeller, and M. a. Geber. 2010. Plant-Soil Water Relations and Species Border of Clarkia xantiana ssp. xantiana (Onagraceae). Int. J. Plant Sci. 171:749–760. University of Chicago Press.
Emery, N. C., M. L. Stanton, and K. J. Rice. 2009. Factors driving distribution limits in an annual plant community. New Phytol. 181:734–747.
Ettema, C. H., and D. A. Wardle. 2002. Spatial soil ecology. Trends Ecol. Evol. 17:177–183.
Ettinger, A., and J. HilleRisLambers. 2017. Competition and facilitation may lead to asymmetric range shift dynamics with climate change. Glob. Chang. Biol., doi: 10.1111/gcb.13649.
Ettinger, A. K., K. R. Ford, and J. HilleRisLambers. 2011. Climate determines upper, but not lower, altitudinal range limits of Pacific Northwest conifers. Ecology 92:1323–1331.
Fierer, N., and R. B. Jackson. 2006. The diversity and biogeography of soil bacterial communities. Proc. Natl. Acad. Sci. U. S. A. 103:626–631.
Fine, P. V. A., I. Mesones, and P. D. Coley. 2004. Herbivores promote habitat specialization by trees in Amazonian forests. Science 305:663–665.
Fisher, R. A. 1930. The Genetical Theory of Natural Selection. Clarendon Press, Oxford.
Fitzpatrick, C. R., Z. Mustafa, and J. Viliunas. 2019. Soil microbes alter plant fitness under competition and drought. J. Evol. Biol. 32:438–450.

Fourcade, Y., A. G. Besnard, and J. Secondi. 2018. Paintings predict the distribution of species, or the challenge of selecting environmental predictors and evaluation statistics. Glob. Ecol. Biogeogr. 27:245–256.
Gallery, R. E., J. W. Dalling, and A. E. Arnold. 2007. Diversity, host affinity, and distribution of seed-infecting fungi: a case study with Cecropia. Ecology 88:582–588.
Garrido, E., A. E. Bennett, J. Fornoni, and S. Y. Strauss. 2010. Variation in arbuscular mycorrhizal fungi colonization modifies the expression of tolerance to above-ground defoliation. J. Ecol. 98:43–49. Blackwell Publishing Ltd.
Gaston, K. J. 2009. Geographic range limits: achieving synthesis. Proc. Biol. Sci. 276:1395–1406.
Geber, M. A., and V. M. Eckhart. 2005. Experimental studies of adaptation in Clarkia xantiana. II. Fitness variation across a subspecies border. Evolution 59:521–531.
Gehrig-Fasel, J., A. Guisan, and N. E. Zimmermann. 2008. Evaluating thermal treeline indicators based on air and soil temperature using an air-to-soil temperature transfer model. Ecol. Modell. 213:345–355. Elsevier.
Gehring, C. A., C. M. Sthultz, L. Flores-Rentería, A. V. Whipple, and T. G. Whitham. 2017. Tree genetics defines fungal partner communities that may confer drought tolerance. Proc. Natl. Acad. Sci. U. S. A. 114:11169–11174. National Acad Sciences.
Gehring, C. A., and T. G. Whitham. 2003. Mycorrhizae-Herbivore Interactions: Population and Community Consequences. Pp. 295–320 *in* M. G. A. van der Heijden and I. R. Sanders, eds. Mycorrhizal Ecology. Springer Berlin Heidelberg, Berlin, Heidelberg.
Godsoe, W., R. Murray, and M. J. Plank. 2015. Information on biotic interactions improves transferability of distribution models. Am. Nat. 185:281–290.
Gohl, D. M., P. Vangay, J. Garbe, A. MacLean, A. Hauge, A. Becker, T. J. Gould, J. B. Clayton, T. J. Johnson, R. Hunter, D. Knights, and K. B. Beckman. 2016. Systematic improvement of amplicon marker gene methods for increased accuracy in microbiome studies. Nat. Biotechnol. 34:942–949.
Gomulkiewicz, R., and R. G. Shaw. 2013. Evolutionary rescue beyond the models. Philos. Trans. R. Soc. Lond. B Biol. Sci. 368:20120093. rstb.royalsocietypublishing.org.
Gould, B., D. A. Moeller, V. M. Eckhart, P. Tiffin, E. Fabio, and M. A. Geber. 2014. Local adaptation and range boundary formation in response to complex environmental gradients across the geographical range of Clarkia xantiana ssp. xantiana. J. Ecol. 102:95–107. Wiley Online Library.
Gravel, D., F. Massol, E. Canard, D. Mouillot, and N. Mouquet. 2011. Trophic theory of island biogeography. Ecol. Lett. 14:1010–1016.
Griffith, T. M., and M. A. Watson. 2006. Is evolution necessary for range expansion? Manipulating reproductive timing of a weedy annual transplanted beyond its range. Am. Nat. 167:153–164. JSTOR.
Grinnell, J. 1917. Field Tests of Theories Concerning Distributional Control. Am. Nat. 51:115–128. The University of Chicago Press.

Güsewell, S. 2004. N : P ratios in terrestrial plants: variation and functional significance: Tansley review. New Phytol. 164:243–266.
Haldane, J. B. 1956. The relation between density regulation and natural selection. Proc. R. Soc. Lond. B Biol. Sci. 145:306–308. royalsocietypublishing.org.
Hao, Y.-Q., M. A. Brockhurst, O. L. Petchey, and Q.-G. Zhang. 2015. Evolutionary rescue can be impeded by temporary environmental amelioration. Ecol. Lett. 18:892–898. Wiley Online Library.
Hargreaves, A. L., K. E. Samis, and C. G. Eckert. 2014. Are species' range limits simply niche limits writ large? A review of transplant experiments beyond the range. Am. Nat. 183:157–173.
Hayat, R., S. Ali, U. Amara, R. Khalid, and I. Ahmed. 2010. Soil beneficial bacteria and their role in plant growth promotion: a review. Ann. Microbiol. 60:579–598. Springer-Verlag.
HilleRisLambers, J., M. A. Harsch, A. K. Ettinger, K. R. Ford, and E. J. Theobald. 2013. How will biotic interactions influence climate change-induced range shifts? Ann. N. Y. Acad. Sci. 1297:112–125.
Hochberg, M. E., and A. R. Ives. 1999. Can natural enemies enforce geographical range limits? Ecography 22:268–276. Blackwell Publishing Ltd.
Hoeksema, J. D., V. B. Chaudhary, C. A. Gehring, N. C. Johnson, J. Karst, R. T. Koide, A. Pringle, C. Zabinski, J. D. Bever, J. C. Moore, and Others. 2010. A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. Ecol. Lett. 13:394–407. Wiley Online Library.
Hoffmann, A. A., and M. W. Blows. 1994. Species borders: ecological and evolutionary perspectives. Trends Ecol. Evol. 9:223–227.
Hoffmann, A. A., R. J. Hallas, J. A. Dean, and M. Schiffer. 2003. Low potential for climatic stress adaptation in a rainforest Drosophila species. Science 301:100–102. science.sciencemag.org.
Holt, R. D., and M. Barfield. 2009. Trophic interactions and range limits: the diverse roles of predation. Proc. Biol. Sci. 276:1435–1442.
Hugoni, M., P. Luis, J. Guyonnet, and F. E. Z. Haichar. 2018. Plant host habitat and root exudates shape fungal diversity. Mycorrhiza 28:451–463.
Hutchinson, G. E. 1957. Concluding remarks. Cold Spring Harbor Symposia on Quantitative Biology 22:415–427.
Islam, M. R., G. Tudryn, R. Bucinell, L. Schadler, and R. C. Picu. 2017. Morphology and mechanics of fungal mycelium. Sci. Rep. 7:13070.
Janzen, D. H. 1970. Herbivores and the Number of Tree Species in Tropical Forests. Am. Nat. 104:501–528. journals.uchicago.edu.
Jasper Wubs, E. R., W. H. van der Putten, M. Bosch, and T. Martijn Bezemer. 2016. Soil inoculation steers restoration of terrestrial ecosystems. Nature Plants 2:16107. Nature Publishing Group.
Johnson, D., J. R. Leake, and D. J. Read. 2001. Novel in-growth core system enables functional studies of grassland mycorrhizal mycelial networks. New Phytol. 152:555–562. Blackwell Science Ltd.

Johnson, N. C., J. H. Graham, and F. A. Smith. 1997. Functioning of mycorrhizal associations along the mutualism–parasitism continuum. New Phytol. Wiley Online Library.
Johnson, N. C., G. W. T. Wilson, M. A. Bowker, J. A. Wilson, and R. M. Miller. 2010. Resource limitation is a driver of local adaptation in mycorrhizal symbioses. Proc. Natl. Acad. Sci. U. S. A. 107:2093–2098.
Jung, S. C., A. Martinez-Medina, J. A. Lopez-Raez, and M. J. Pozo. 2012. Mycorrhiza-induced resistance and priming of plant defenses. J. Chem. Ecol. 38:651–664.
Katz, D. S. W. 2016. The effects of invertebrate herbivores on plant population growth: a meta-regression analysis. Oecologia 182:43–53. Springer.
Katz, D. S. W., and I. Ibáñez. 2017. Differences in biotic interactions across range edges have only minor effects on plant performance. J. Ecol. 105:321–331. Wiley Online Library.
Kawecki, T. J. 2008. Adaptation to Marginal Habitats. Annu. Rev. Ecol. Evol. Syst. 39:321–342. Annual Reviews.
Kennedy, P. G., S. Hortal, S. E. Bergemann, and T. D. Bruns. 2007. Competitive interactions among three ectomycorrhizal fungi and their relation to host plant performance. J. Ecol. 95:1338–1345.
Keymer, D. P., and R. A. Lankau. 2017. Disruption of plant–soil–microbial relationships influences plant growth. J. Ecol. Wiley Online Library.
Kirkpatrick, M., and N. H. Barton. 1997. Evolution of a species' range. Am. Nat. 150:1–23.
Kirkpatrick, M., and S. Peischl. 2013. Evolutionary rescue by beneficial mutations in environments that change in space and time. Philos. Trans. R. Soc. Lond. B Biol. Sci. 368:20120082.
Klironomos, J. N. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417:67–70. nature.com.
Klironomos, J. N. 2003. Variation in plant responses to native and exotic arbuscular mycorrhizal fungi. Ecology 84:2292–2301. Ecological Society of America.
Knight, R., A. Vrbanac, B. C. Taylor, A. Aksenov, C. Callewaert, J. Debelius, A. Gonzalez, T. Kosciolek, L.-I. McCall, D. McDonald, A. V. Melnik, J. T. Morton, J. Navas, R. A. Quinn, J. G. Sanders, A. D. Swafford, L. R. Thompson, A. Tripathi, Z. Z. Xu, J. R. Zaneveld, Q. Zhu, J. G. Caporaso, and P. C. Dorrestein. 2018. Best practices for analysing microbiomes. Nat. Rev. Microbiol. 16:410–422.
Krimmel, B., and I. S. Pearse. 2016. Tolerance and phenological avoidance of herbivory in tarweed species. Ecology 97:1357–1363.
Lafleur, B., D. Paré, A. D. Munson, and Y. Bergeron. 2010. Response of northeastern North American forests to climate change: Will soil conditions constrain tree species migration? Environ. Rev. 18:279–289. NRC Research Press.
Lamb, E. G., S. Han, B. D. Lanoil, G. H. R. Henry, M. E. Brummell, S. Banerjee, and S. D. Siciliano. 2011. A High Arctic soil ecosystem resists long-term environmental manipulations. Glob. Chang. Biol. 17:3187–3194. Wiley Online Library.
Lankau, R. A. 2013. Species invasion alters local adaptation to soil communities in a native plant. Ecology 94:32–40. Wiley Online Library.

Lankau, R. A., and D. P. Keymer. 2016. Ectomycorrhizal fungal richness declines towards the host species' range edge. Mol. Ecol. 25:3224–3241.
Lankau, R. A., and D. P. Keymer. 2018. Simultaneous adaptation and maladaptation of tree populations to local rhizosphere microbial communities at different taxonomic scales. New Phytol. 217:1267–1278. Wiley Online Library.
Lau, J. A., and J. T. Lennon. 2012. Rapid responses of soil microorganisms improve plant fitness in novel environments. Proc. Natl. Acad. Sci. U. S. A. 109:14058–14062.
Lau, J. A., A. C. McCall, K. F. Davies, J. K. McKay, and J. W. Wright. 2008. Herbivores and edaphic factors constrain the realized niche of a native plant. Ecology 89:754–762.
Lazarus, B. E., J. H. Richards, V. P. Claassen, R. E. O'Dell, and M. A. Ferrell. 2011. Species specific plant-soil interactions influence plant distribution on serpentine soils. Plant Soil 342:327–344.
Lee-Yaw, J. A., H. M. Kharouba, M. Bontrager, C. Mahony, A. M. Csergő, A. M. E. Noreen, Q. Li, R. Schuster, and A. L. Angert. 2016. A synthesis of transplant experiments and ecological niche models suggests that range limits are often niche limits. Ecol. Lett. 19:710–722. Wiley Online Library.
Levin, D. A. 2006. Flowering Phenology in Relation to Adaptive Radiation. Syst. Bot. 31:239–246. The American Society of Plant Taxonomists.
Levin, D. A., and K. Clay. 1984. DYNAMICS OF SYNTHETIC PHLOX DRUMMONDII POPULATIONS AT THE SPECIES MARGIN. Am. J. Bot. 71:1040–1050. Wiley Online Library.
Levin, P. A., and E. R. Angert. 2015. Small but Mighty: Cell Size and Bacteria. Cold Spring Harb. Perspect. Biol. 7:a019216.
Lewis, H., and M. E. Lewis. 1955. The genus Clarkia. CA, USA: University of California Press.
Louda, S. M. 1982. Distribution Ecology: Variation in Plant Recruitment over a Gradient in Relation to Insect Seed Predation. Ecol. Monogr. 52:25–41. Ecological Society of America.
Louthan, A. M., D. F. Doak, and A. L. Angert. 2015. Where and When do Species Interactions Set Range Limits? Trends Ecol. Evol. 30:780–792.
Louthan, A. M., D. F. Doak, J. R. Goheen, T. M. Palmer, and R. M. Pringle. 2013. Climatic stress mediates the impacts of herbivory on plant population structure and components of individual fitness. J. Ecol. 101:1074–1083. Wiley Online Library.
Lugtenberg, B., and F. Kamilova. 2009. Plant-growth-promoting rhizobacteria. Annu. Rev. Microbiol. 63:541–556.
MacArthur, R. H. 1972. Geographical Ecology: Patterns in the Distribution of Species. Harper and Row, New York.
Maestre, F. T., M. Delgado-Baquerizo, T. C. Jeffries, D. J. Eldridge, V. Ochoa, B. Gozalo, J. L. Quero, M. García-Gómez, A. Gallardo, W. Ulrich, M. A. Bowker, T. Arredondo, C. Barraza-Zepeda, D. Bran, A. Florentino, J. Gaitán, J. R. Gutiérrez, E. Huber-Sannwald, M. Jankju, R. L. Mau, M. Miriti, K. Naseri, A. Ospina, I. Stavi, D. Wang, N. N. Woods, X. Yuan, E. Zaady, and B. K. Singh. 2015. Increasing aridity reduces soil microbial diversity and abundance in global drylands. Proc. Natl. Acad. Sci. U. S. A. 112:15684–15689. National Acad Sciences.

Manzanedo, R. D., F. R. Schanz, M. Fischer, and E. Allan. 2018. Fagus sylvatica seedlings show provenance differentiation rather than adaptation to soil in a transplant experiment. BMC Ecol. 18:42.
Mayr, E., E. Mayr, E. Mayr, and E. Mayr. 1963. Animal species and evolution. coleoguy.github.io.
Mengel, K., E. A. Kirkby, H. Kosegarten, and T. Appel (eds). 2001. Principles of Plant Nutrition. Springer, Dordrecht.
Milner, J. M., S. D. Albon, A. W. Illius, J. M. Pemberton, and T. H. Clutton-Brock. 1999. Repeated selection of morphometric traits in the Soay sheep on St Kilda. J. Anim. Ecol. 68:472–488.
Miransari, M. 2010. Contribution of arbuscular mycorrhizal symbiosis to plant growth under different types of soil stress. Plant Biol. 12:563–569.
Mlynarek, J. J., C. E. Moffat, S. Edwards, A. L. Einfeldt, A. Heustis, R. Johns, M. MacDonnell, D. S. Pureswaran, D. T. Quiring, Z. Shibel, and S. B. Heard. 2017. Enemy escape: A general phenomenon in a fragmented literature? FACETS 2:1015–1044.
Moeller, D. A. 2006. Geographic structure of pollinator communities, reproductive assurance, and the evolution of self-pollination. Ecology 87:1510–1522. Wiley Online Library.
Moeller, D. A. 2005. Pollinator community structure and sources of spatial variation in plant–pollinator interactions in Clarkia xantiana ssp. xantiana. Oecologia 142:28–37. Springer-Verlag.
Moeller, D. A., M. A. Geber, V. M. Eckhart, and P. Tiffin. 2012. Reduced pollinator service and elevated pollen limitation at the geographic range limit of an annual plant. Ecology 93:1036–1048. Wiley Online Library.
Moeller, D. A., M. A. Geber, and P. Tiffin. 2011. Population genetics and the evolution of geographic range limits in an annual plant. Am. Nat. 178 Suppl 1:S44–57.
Mommer, L., T. E. Cotton, J. M. Raaijmakers, A. J. Termorshuizen, J. Ruijven, M. Hendriks, S. Q. Rijssel, J. E. Mortel, J. W. Paauw, E. G. Schijlen, and Others. 2018. Lost in diversity: the interactions between soil-borne fungi, biodiversity and plant productivity. New Phytol. Wiley Online Library.
Nadeau, C. P., M. C. Urban, and J. R. Bridle. 2017. Climates Past, Present, and Yet-to-Come Shape Climate Change Vulnerabilities. Trends Ecol. Evol. 32:786–800. Elsevier.
Nelson, J. M. 2017. Diversity and Effects of the Fungal Endophytes of the Liverwort Marchantia polymorpha. Duke University.
Nguyen, N. H., D. Smith, K. Peay, and P. Kennedy. 2015. Parsing ecological signal from noise in next generation amplicon sequencing. New Phytol. 205:1389–1393.
Noguez, A. M., H. T. Arita, A. E. Escalante, L. J. Forney, F. García-Oliva, and V. Souza. 2005. Microbial macroecology: highly structured prokaryotic soil assemblages in a tropical deciduous forest: Prokaryotic macroecology. Glob. Ecol. Biogeogr. 14:241–248.
Nuñez, M. A., T. R. Horton, and D. Simberloff. 2009. Lack of belowground mutualisms hinders Pinaceae invasions. Ecology 90:2352–2359. Wiley Online Library.

O'Brien, E. K., M. Higgie, A. Reynolds, A. A. Hoffmann, and J. R. Bridle. 2017. Testing for local adaptation and evolutionary potential along altitudinal gradients in rainforest Drosophila: beyond laboratory estimates. Glob. Chang. Biol. 23:1847–1860.
Osborne, O. G., R. De-Kayne, M. I. Bidartondo, I. Hutton, W. J. Baker, C. G. N. Turnbull, and V. Savolainen. 2018. Arbuscular mycorrhizal fungi promote coexistence and niche divergence of sympatric palm species on a remote oceanic island. New Phytol. 217:1254–1266.
Pain, R. E., R. G. Shaw, and S. N. Sheth. 2018. Detrimental effects of rhizobial inoculum early in the life of partridge pea, Chamaecrista fasciculata. Am. J. Bot. 17:1265.
Pake, C. E., and D. L. Venable. 1996. Seed Banks in Desert Annuals: Implications for Persistence and Coexistence in Variable Environments. Ecology 77:1427–1435.
Palm, C., P. Sanchez, S. Ahamed, and A. Awiti. 2007. Soils: A Contemporary Perspective. Annu. Rev. Environ. Resour. 32:99–129. Annual Reviews.
Palmer, J. M., M. A. Jusino, M. T. Banik, and D. L. Lindner. 2018. Non-biological synthetic spike-in controls and the AMPtk software pipeline improve mycobiome data. PeerJ 6:e4925.
Parker, I. M., and G. S. Gilbert. 2004. The Evolutionary Ecology of Novel Plant-Pathogen Interactions. Annu. Rev. Ecol. Evol. Syst. 35:675–700. Annual Reviews.
Parker, M. A. 2001. Mutualism as a constraint on invasion success for legumes and rhizobia. Divers. Distrib. 7:125–136.
Parmesan, C., and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature 421:37–42.
Passioura, J. B. 1991. Soil structure and plant growth. Soil Res. 29:717–728. CSIRO PUBLISHING.
Pearson, R. G., and T. P. Dawson. 2003. Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? Glob. Ecol. Biogeogr. 12:361–371. Wiley Online Library.
Peay, K. G., P. G. Kennedy, S. J. Davies, S. Tan, and T. D. Bruns. 2010. Potential link between plant and fungal distributions in a dipterocarp rainforest: community and phylogenetic structure of tropical ectomycorrhizal fungi across a plant and soil ecotone. New Phytol. 185:529–542.
Pettengill, J. B., and D. A. Moeller. 2012a. Phylogeography of speciation: allopatric divergence and secondary contact between outcrossing and selfing Clarkia. Mol. Ecol. 21:4578–4592.
Pettengill, J. B., and D. A. Moeller. 2012b. Tempo and mode of mating system evolution between incipient Clarkia species. Evolution 66:1210–1225. Wiley Online Library.
Pickles, B. J., B. D. Twieg, G. A. O'Neill, W. W. Mohn, and S. W. Simard. 2015. Local adaptation in migrated interior Douglas-fir seedlings is mediated by ectomycorrhizas and other soil factors. New Phytol. 207:858–871. Wiley Online Library.
Pigot, A. L., and J. A. Tobias. 2013. Species interactions constrain geographic range expansion over evolutionary time. Ecol. Lett. 16:330–338.
Pilson, D. 2000. Herbivory and natural selection on flowering phenology in wild sunflower, Helianthus annuus. Oecologia 122:72–82. Springer.

Polechova, J. 2018. Is the sky the limit? On the expansion threshold of a species' range. PLoS Biol. 16:e2005372. Public Library of Science.
Polechová, J., and N. H. Barton. 2015. Limits to adaptation along environmental gradients. Proc. Natl. Acad. Sci. U. S. A. 112:6401–6406.
Poorter, H., F. Fiorani, R. Pieruschka, T. Wojciechowski, W. H. van der Putten, M. Kleyer, U. Schurr, and J. Postma. 2016. Pampered inside, pestered outside? Differences and similarities between plants growing in controlled conditions and in the field. New Phytol. 212:838–855.
Prober, S. M., J. W. Leff, S. T. Bates, E. T. Borer, J. Firn, W. S. Harpole, E. M. Lind, E. W. Seabloom, P. B. Adler, J. D. Bakker, E. E. Cleland, N. M. DeCrappeo, E. DeLorenze, N. Hagenah, Y. Hautier, K. S. Hofmockel, K. P. Kirkman, J. M. H. Knops, K. J. La Pierre, A. S. MacDougall, R. L. McCulley, C. E. Mitchell, A. C. Risch, M. Schuetz, C. J. Stevens, R. J. Williams, and N. Fierer. 2015. Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. Ecol. Lett. 18:85–95.
Quinn, R. D. 1986. Mammalian herbivory and resilience in Mediterranean-climate ecosystems. *in* Bernard Dell A J M Hopkins, ed. Resilience in Mediterranean type ecosystems.
Reed, D. H., and R. Frankham. 2001. How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. Evolution 55:1095–1103. Wiley Online Library.
Reinhart, K. O., T. Tytgat, W. H. Van der Putten, and K. Clay. 2010. Virulence of soil-borne pathogens and invasion by Prunus serotina. New Phytol. 186:484–495. Wiley Online Library.
Revillini, D., C. A. Gehring, and N. C. Johnson. 2016. The role of locally adapted mycorrhizas and rhizobacteria in plant-soil feedback systems. Funct. Ecol. 30:1086–1098.
Rinnan, R., A. Michelsen, E. Bååth, and S. Jonasson. 2007. Fifteen years of climate change manipulations alter soil microbial communities in a subarctic heath ecosystem. Glob. Chang. Biol. 13:28–39.
Rúa, M. A., A. Antoninka, P. M. Antunes, V. B. Chaudhary, C. Gehring, L. J. Lamit, B. J. Piculell, J. D. Bever, C. Zabinski, J. F. Meadow, M. J. Lajeunesse, B. G. Milligan, J. Karst, and J. D. Hoeksema. 2016. Home-field advantage? evidence of local adaptation among plants, soil, and arbuscular mycorrhizal fungi through meta-analysis. BMC Evol. Biol. 16:122.
Rumpf, S. B., K. Hülber, G. Klonner, D. Moser, M. Schütz, J. Wessely, W. Willner, N. E. Zimmermann, and S. Dullinger. 2018. Range dynamics of mountain plants decrease with elevation. Proc. Natl. Acad. Sci. U. S. A. 115:1848–1853.
Sæther, B.-E., and S. Engen. 2015. The concept of fitness in fluctuating environments. Trends Ecol. Evol. 30:273–281. Elsevier.
Samis, K. E., and C. G. Eckert. 2009. Ecological correlates of fitness across the northern geographic range limit of a Pacific Coast dune plant. Ecology 90:3051–3061. Wiley Online Library.
Sarmiento, C., P.-C. Zalamea, J. W. Dalling, A. S. Davis, S. M. Stump, J. M. U'Ren, and A. E. Arnold. 2017. Soilborne fungi have host affinity and host-specific effects on

seed germination and survival in a lowland tropical forest. Proc. Natl. Acad. Sci. U. S. A. 114:11458–11463.
Schultz, P. A., R. Michael Miller, J. D. Jastrow, C. V. Rivetta, and J. D. Bever. 2001. Evidence of a mycorrhizal mechanism for the adaptation of Andropogon gerardii (Poaceae) to high- and low-nutrient prairies. Am. J. Bot. 88:1650–1656.
Scully, A. E., S. Fisher, D. A. W. Miller, and D. H. Thornton. 2018. Influence of biotic interactions on the distribution of Canada lynx (Lynx canadensis) at the southern edge of their range. J. Mammal., doi: 10.1093/jmammal/gyy053.
Sexton, J. P., P. J. McIntyre, A. L. Angert, and K. J. Rice. 2009. Evolution and Ecology of Species Range Limits. Annu. Rev. Ecol. Evol. Syst. 40:415–436.
Sexton, J. P., S. Y. Strauss, and K. J. Rice. 2011. Gene flow increases fitness at the warm edge of a species' range. Proc. Natl. Acad. Sci. U. S. A. 108:11704–11709.
Shaw, R. G., C. J. Geyer, S. Wagenius, H. H. Hangelbroek, and J. R. Etterson. 2008. Unifying Life-History Analyses for Inference of Fitness and Population Growth. Am. Nat. 172:E35–E47.
Sherrard, M. E., and H. Maherali. 2012. Local adaptation across a fertility gradient is influenced by soil biota in the invasive grass, Bromus inermis. Evol. Ecol. 26:529–544. Springer.
Siepielski, A. M., J. DiBattista, and S. M. Carlson. 2018. It's about time: the temporal dynamics of phenotypic selection in the wild. Ecol. Lett. 1261–1276. John Wiley & Sons, Ltd (10.1111).
Smith, D. S., J. A. Schweitzer, P. Turk, J. K. Bailey, S. C. Hart, S. M. Shuster, and T. G. Whitham. 2012. Soil-mediated local adaptation alters seedling survival and performance. Plant Soil 352:243–251. Springer Netherlands.
Stahl, P. D., and W. K. Smith. 1984. Effects of Different Geographic Isolates of Glomus on the Water Relations of Agropyron Smithii. Mycologia 76:261–267. Taylor & Francis.
Staniczenko, P. P. A., K. B. Suttle, and R. G. Pearson. 2018. Negative biotic interactions drive predictions of distributions for species from a grassland community. Biol. Lett. 14.
Stanton-Geddes, J., and C. G. Anderson. 2011. Does a facultative mutualism limit species range expansion? Oecologia 167:149–155.
Stanton-Geddes, J., R. G. Shaw, and P. Tiffin. 2012a. Interactions between soil habitat and geographic range location affect plant fitness. PLoS One 7:e36015.
Stanton-Geddes, J., P. Tiffin, and R. G. Shaw. 2012b. Role of climate and competitors in limiting fitness across range edges of an annual plant. Ecology 93:1604–1613.
Su, Y.-Y., L.-D. Guo, and K. D. Hyde. 2010. Response of endophytic fungi of Stipa grandis to experimental plant function group removal in Inner Mongolia steppe, China. Fungal Divers. 43:93–101.
Svenning, J.-C., S. Normand, and F. Skov. 2008. Postglacial dispersal limitation of widespread forest plant species in nemoral Europe. Ecography 31:316–326. Blackwell Publishing Ltd.
Svenning, J.-C., and F. Skov. 2007. Could the tree diversity pattern in Europe be generated by postglacial dispersal limitation? Ecol. Lett. 10:453–460. Wiley Online Library.

Tedersoo, L., M. Bahram, S. Põlme, U. Kõljalg, N. S. Yorou, R. Wijesundera, L. Villarreal Ruiz, A. M. Vasco-Palacios, P. Q. Thu, A. Suija, M. E. Smith, C. Sharp, E. Saluveer, A. Saitta, M. Rosas, T. Riit, D. Ratkowsky, K. Pritsch, K. Põldmaa, M. Piepenbring, C. Phosri, M. Peterson, K. Parts, K. Pärtel, E. Otsing, E. Nouhra, A. L. Njouonkou, R. H. Nilsson, L. N. Morgado, J. Mayor, T. W. May, L. Majuakim, D. J. Lodge, S. S. Lee, K.-H. Larsson, P. Kohout, K. Hosaka, I. Hiiesalu, T. W. Henkel, H. Harend, L.-D. Guo, A. Greslebin, G. Grelet, J. Geml, G. Gates, W. Dunstan, C. Dunk, R. Drenkhan, J. Dearnaley, A. De Kesel, T. Dang, X. Chen, F. Buegger, F. Q. Brearley, G. Bonito, S. Anslan, S. Abell, and K. Abarenkov. 2014. Fungal biogeography. Global diversity and geography of soil fungi. Science 346:1256688.

Templeton, A. R., and D. A. Levin. 1979. Evolutionary Consequences of Seed Pools. Am. Nat. 114:232–249. The University of Chicago Press.

Teste, F. P., J. Karst, M. D. Jones, S. W. Simard, and D. M. Durall. 2006. Methods to control ectomycorrhizal colonization: effectiveness of chemical and physical barriers. Mycorrhiza 17:51–65.

Thrall, P. H., M. E. Hochberg, J. J. Burdon, and J. D. Bever. 2007. Coevolution of symbiotic mutualists and parasites in a community context. Trends Ecol. Evol. 22:120–126.

Thuiller, W. 2013. On the importance of edaphic variables to predict plant species distributions - limits and prospects. J. Veg. Sci. 24:591–592.

Thuiller, W., S. Lavorel, G. Midgley, S. Lavergne, and T. Rebelo. 2004. Relating plant traits and species distributions along bioclimatic gradients for 88 Leucadendron taxa. Ecology 85:1688–1699. Ecological Society of America.

Tian, L., S. Shi, L. Ma, F. Nasir, X. Li, L.-S. P. Tran, and C. Tian. 2018. Co-evolutionary associations between root-associated microbiomes and root transcriptomes in wild and cultivated rice varieties. Plant Physiol. Biochem. 128:134–141.

Urban, M. C., G. Bocedi, A. P. Hendry, J.-B. Mihoub, G. Pe'er, A. Singer, J. R. Bridle, L. G. Crozier, L. De Meester, W. Godsoe, A. Gonzalez, J. J. Hellmann, R. D. Holt, A. Huth, K. Johst, C. B. Krug, P. W. Leadley, S. C. F. Palmer, J. H. Pantel, A. Schmitz, P. A. Zollner, and J. M. J. Travis. 2016. Improving the forecast for biodiversity under climate change. Science 353:aad8466. American Association for the Advancement of Science.

Van Der Heijden, M. G. A., R. D. Bardgett, and N. M. Van Straalen. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol. Lett. 11:296–310. Blackwell Publishing Ltd.

van der Heijden, M. G. A., S. de Bruin, L. Luckerhoff, R. S. P. van Logtestijn, and K. Schlaeppi. 2016. A widespread plant-fungal-bacterial symbiosis promotes plant biodiversity, plant nutrition and seedling recruitment. ISME J. 10:389–399.

van der Heijden, M. G. A., J. N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, and I. R. Sanders. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69–72.

van der Putten, W. H. 2012. Climate Change, Aboveground-Belowground Interactions, and Species' Range Shifts. Annu. Rev. Ecol. Evol. Syst. 43:365–383. Annual Reviews.

van der Putten, W. H., M. Macel, and M. E. Visser. 2010. Predicting species distribution and abundance responses to climate change: why it is essential to include biotic interactions across trophic levels. Philos. Trans. R. Soc. Lond. B Biol. Sci. 365:2025–2034. rstb.royalsocietypublishing.org.

Visser, M. E., and L. J. Holleman. 2001. Warmer springs disrupt the synchrony of oak and winter moth phenology. Proc. Biol. Sci. 268:289–294.

Volis, S. 2007. Correlated patterns of variation in phenology and seed production in populations of two annual grasses along an aridity gradient. Evol. Ecol. 21:381–393. Kluwer Academic Publishers.

Wagner, M. R., D. S. Lundberg, D. Coleman-Derr, S. G. Tringe, J. L. Dangl, and T. Mitchell-Olds. 2014. Natural soil microbes alter flowering phenology and the intensity of selection on flowering time in a wild Arabidopsis relative. Ecol. Lett. 17:717–726.

Waldrop, M. P., and M. K. Firestone. 2006. Response of microbial community composition and function to soil climate change. Microb. Ecol. 52:716–724.

Walthert, L., and E. S. Meier. 2017. Tree species distribution in temperate forests is more influenced by soil than by climate. Ecol. Evol. 7:9473–9484.\

Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73:5261–5267.

Waraich, E. A., R. Ahmad, and M. Y. Ashraf. 2011. Role of Mineral Nutrition in Alleviation of Drought Stress in Plants. Aust. J. Crop Sci. 5:764. Southern Cross Publishers.

Weir, J. T., M. S. Faccio, P. Pulido-Santacruz, A. O. Barrera-Guzmán, and A. Aleixo. 2015. Hybridization in headwater regions, and the role of rivers as drivers of speciation in Amazonian birds. Evolution 69:1823–1834.

Whitlock, M. C. 2004. Selection and drift in metapopulations. Ecology, genetics and evolution of metapopulations. Elsevier.

Wisz, M. S., J. Pottier, W. D. Kissling, L. Pellissier, J. Lenoir, C. F. Damgaard, C. F. Dormann, M. C. Forchhammer, J.-A. Grytnes, A. Guisan, R. K. Heikkinen, T. T. Høye, I. Kühn, M. Luoto, L. Maiorano, M.-C. Nilsson, S. Normand, E. Öckinger, N. M. Schmidt, M. Termansen, A. Timmermann, D. A. Wardle, P. Aastrup, and J.-C. Svenning. 2013. The role of biotic interactions in shaping distributions and realised assemblages of species: implications for species distribution modelling. Biol. Rev. Camb. Philos. Soc. 88:15–30.

Wolfe, B. E., B. C. Husband, and J. N. Klironomos. 2005. Effects of a belowground mutualism on an aboveground mutualism. Ecol. Lett. Wiley Online Library.

Wolfe, B. E., D. L. Mummey, M. C. Rillig, and J. N. Klironomos. 2007. Small-scale spatial heterogeneity of arbuscular mycorrhizal fungal abundance and community composition in a wetland plant community. Mycorrhiza 17:175–183. Springer.

Wubs, E. R. J., W. H. van der Putten, S. R. Mortimer, G. W. Korthals, H. Duyts, R. Wagenaar, and T. M. Bezemer. 2019. Single introductions of soil biota and plants generate long-term legacies in soil and plant community assembly. Ecol. Lett., doi: 10.1111/ele.13271.

Yang, H., Y. Yuan, Q. Zhang, J. Tang, Y. Liu, and X. Chen. 2011. Changes in soil organic carbon, total nitrogen, and abundance of arbuscular mycorrhizal fungi along a large-scale aridity gradient. Catena 87:70–77.

Yang, J., J. W. Kloepper, and C.-M. Ryu. 2009. Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci. 14:1–4.

Zak, D. R., W. E. Holmes, D. C. White, A. D. Peacock, and D. Tilman. 2003. PLANT DIVERSITY, SOIL MICROBIAL COMMUNITIES, AND ECOSYSTEM FUNCTION: ARE THERE ANY LINKS? Ecology 84:2042–2050.

Zamin, T. J., M. S. Bret-Harte, and P. Grogan. 2014. Evergreen shrubs dominate responses to experimental summer warming and fertilization in Canadian mesic low arctic tundra. J. Ecol. 102:749–766.

Appendix 1

A: Stem translocations

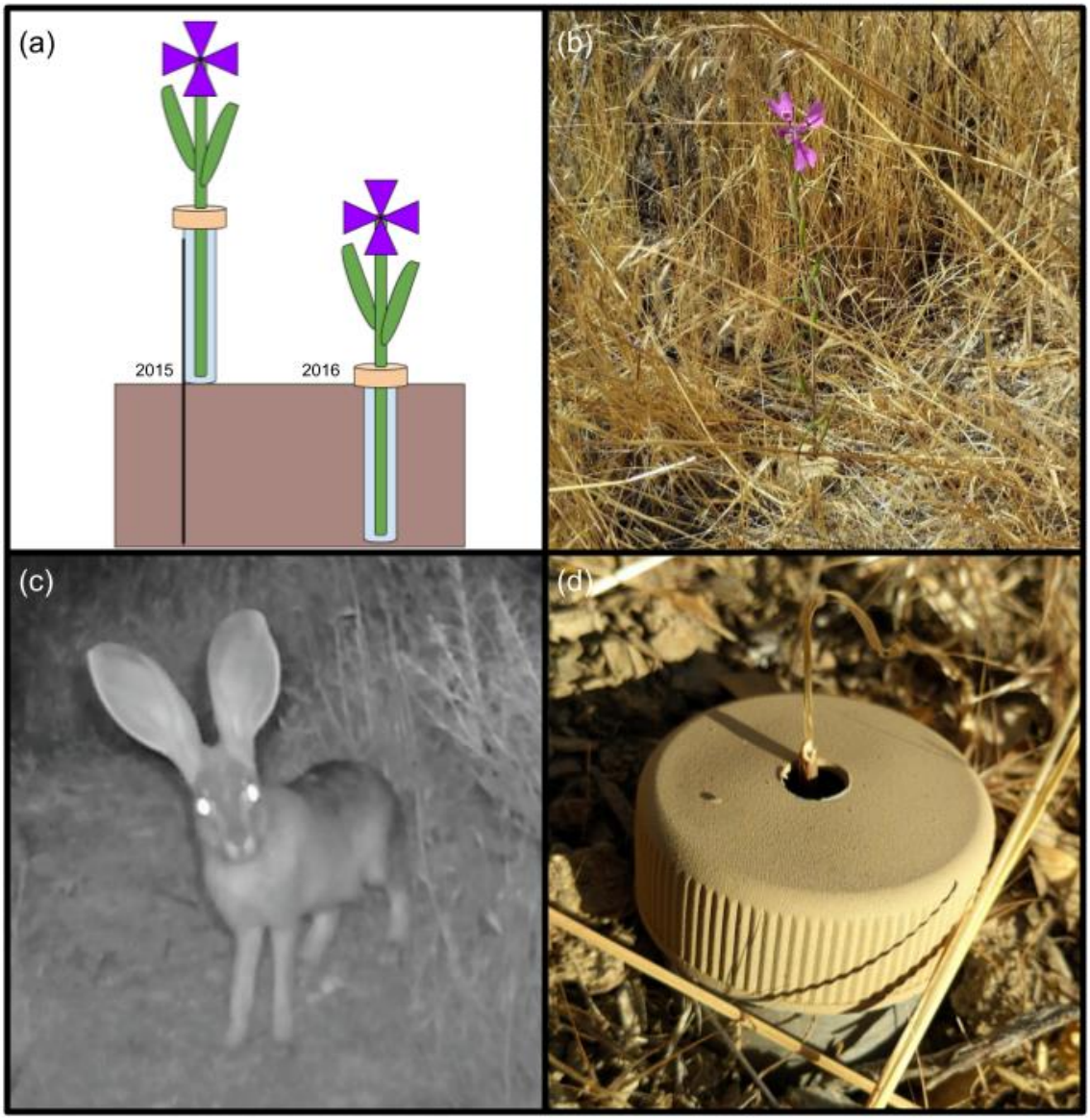

Figure A1. Stem-translocation experimental protocol and observations. (a) illustrates how *xantiana* stems were translocated in the 2015-2016 field experiments using small plastic tubes; presentation of stems differed slightly between years. (b) shows translocated *xantiana* stem in the field. (c) shows image of *Lepus californicus*, a primary herbivore of *xantiana*, captured at one of the experimental sites with a motion-triggered game camera. (d) shows example of fatal herbivory commonly observed on translocated (and natural) *xantiana* stems.

A.1: Herbivory on natural *xantiana* during stem translocation experiment

During the 2015 stem translocation experiment, at the five sites within the natural distribution of *xantiana* we also followed naturally occurring plants within the vicinity of the experiment to determine whether geographic patterns of herbivory on experimental plants mimicked that on natural plants. Along two to four transects at each site, naturally occurring *xantiana* plants (28 to 40 at each site) were marked with small metal collars (a piece of wire encircling the stem ~2 cm off the ground) and followed throughout the experiment to record herbivory. Figure S2 compares recorded herbivory rates on translocated and natural plants; herbivory on translocated stems closely reflected herbivory on natural *xantiana* at four of five sites, with herbivory rates on natural and translocated *xantiana* differing by less than five percent. At one site (Sawmill Road), herbivory rates on translocated stems were much higher than on natural plants (0.81 vs 0.05). We compared logistic regressions of herbivory on easting (see Methods) including and excluding this site; both models gave qualitatively similar ANOVA results, and predicted probabilities of herbivory did not change substantially when the site was excluded, though the point of inflection for the logistic curve drew closer to the range limit (Fig. S3); thus, we included the site for the analyses reported in the main text.

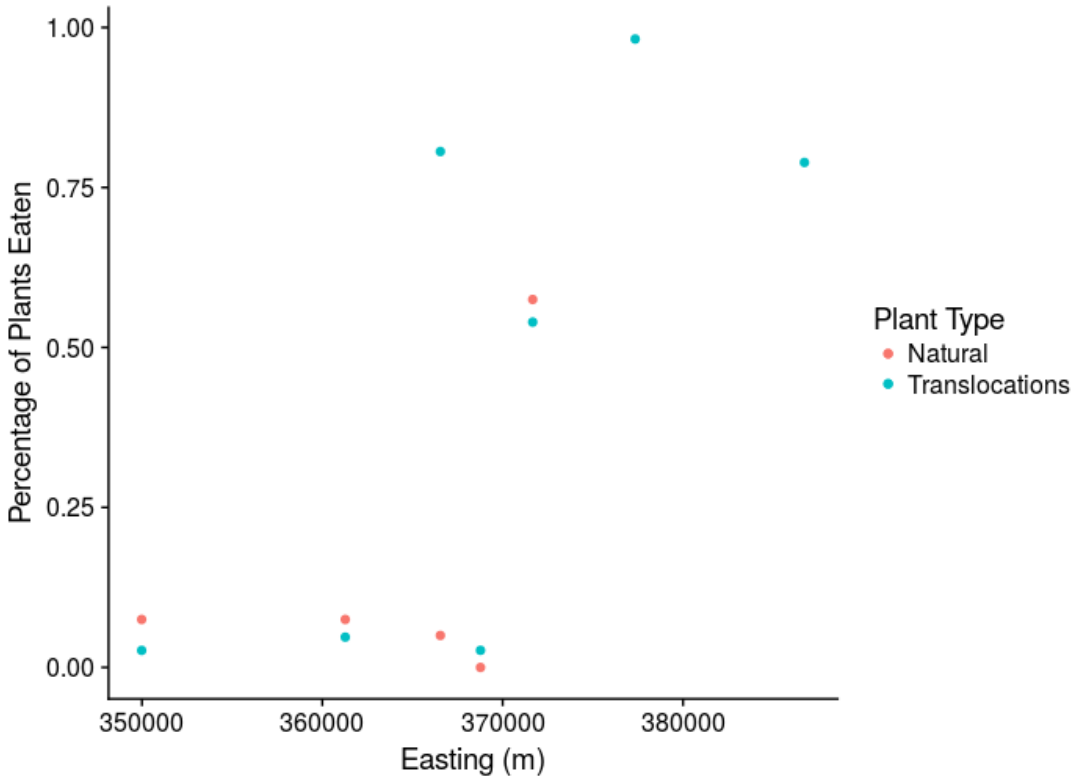

Figure A2. Comparison of herbivory rates on natural plants and translocated stems of *xantiana* at the five sites within *xantiana*'s natural range in 2015.

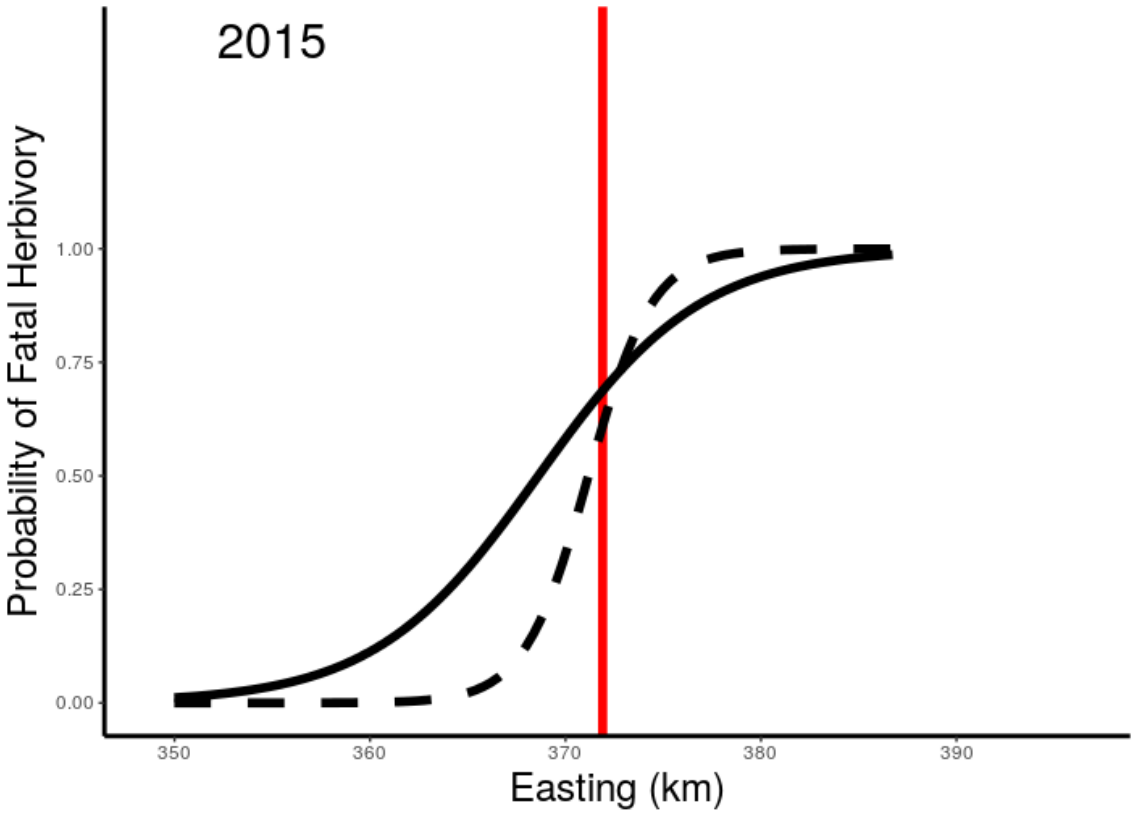

Figure A3. Comparison of conditional predicted probability functions of herbivory given easting, with all 2015 sites included (solid line), and excluding Sawmill Road (dashed line).

Table A1. Results of Type II Analysis of Deviance using likelihood ratio tests for the logistic regression model, Herbivory ~ Easting * Census Round * Source + Easting:Census Round:Transect, using data from stem translocation experiments in 2015 and 2016. In 2016, all stems were from the same Source, so this term was not included, and Easting was included as a quadratic term after comparing BIC scores with linear, quadratic, and cubic terms. Non-significant interactions are not shown.

2015			**2016**		
term	*df*	χ^2	*term*	*df*	χ^2
Easting	1	498.2***	**Easting^2**	1	86.3***
Round	3	58.7***	**Round**	2	12.3**
Source	1	0.36	**Easting^2:Round**	2	0.9
Easting: Round	3	41.54***	**Round:Easting: Transect**	6	27.1***
————————			————————		
Nagelkerke's Pseudo R^2		0.49	Nagelkerke's Pseudo R^2		0.33

*, **, and *** indicate P values lower than 0.05, 0.01, and 0.001, respectively.

B: Reciprocal transplant: herbivory summary and fitness simulations

B.1: Temporal and spatial variation in herbivory during the reciprocal transplant

Table B1. Summary of fatal mammalian herbivory on *xantiana* and *parviflora* at three reciprocal transplant sites in 1997-1998 (Wet Year) and 1998-1999 (Dry Year).

Year	Site	Subspecies	Number of germinants	Number of plants eaten	Percent herbivory
Wet	Center	*parviflora*	896	135	0.15
Wet	Center	*xantiana*	445	68	0.15
Wet	Edge	*parviflora*	906	72	0.08
Wet	Edge	*xantiana*	470	158	0.34
Wet	Beyond Edge	*parviflora*	506	95	0.19
Wet	Beyond Edge	*xantiana*	291	156	0.54
Dry	Center	*parviflora*	597	28	0.05
Dry	Center	*xantiana*	303	8	0.03
Dry	Edge	*parviflora*	439	6	0.01
Dry	Edge	*xantiana*	210	2	0.01
Dry	Beyond Edge	*parviflora*	170	6	0.04
Dry	Beyond Edge	*xantiana*	179	5	0.03

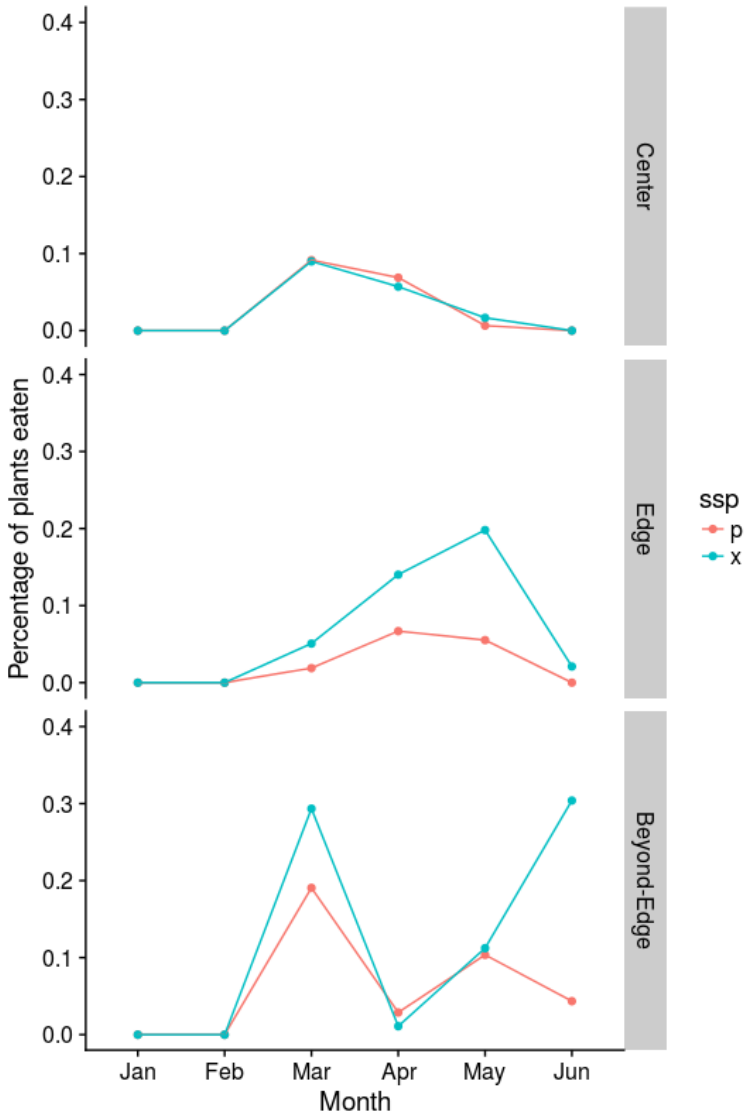

Figure B1. Temporal trends in fatal mammalian herbivory for both subspecies in Center, Edge, and Beyond-Edge sites during the wet year (1997-1998) of the transplant experiment.

B.2: Predicting fitness of eaten plants

To predict potential fitness values for plants eaten during the transplant experiment, we first modeled the probability of a plant successfully reproducing (at least one seed produced) using logistic regression with year, site, subspecies, the plant's size in February, and all interactions (plus block nested within year and site, and population nested within subspecies) as terms, using only plants that did not experience herbivory. This model predicted probability of producing seed very well (Nagelkerke's $R^2 = 0.75$), and we used it to predict the probability of seed production for all eaten plants (rounding model predicted probabilities to one or zero).

Then, using plants that were not eaten and produced seed in the experiment, we used a linear model to model seed output (log transformed) of plants based on year, site, subspecies, the plant's size in February, and all interactions (plus block nested within year and site, and population nested within subspecies) as terms. This model predicted seed set fairly well (multiple $R^2 = 0.61$; adjusted $R^2 = 0.55$). We used size at the February census in these models because this was the latest census that preceded all herbivory events; "size" in this context and all others below was calculated as the product of plant leaf number and average leaf length.

We then used those models to predict point estimates of those two lifetime fitness components for all plants in the data set. Then, we created 100 simulation data sets, where for each plant in each simulation we assigned a reproduction and seed set value. The reproduction score was based on a random pull from a binomial distribution $B(1, p)$, with the probability parameter p equal to that plant's point estimate for reproduction. Seed set for a plant was pulled from the normal distribution $N(\mu, \sigma^2)$, with μ equal to the plant's seed set estimate, and σ^2equal to the estimated standard deviation of the residuals from the predictive linear model of seed set. Thus, for that simulation run, a given plant's fitness estimate would be the product of its reproductive score and seed set estimate.

Since the predictive models are based on field data, this fitness estimate for eaten plants reflects plant responses to all aspects of the environment beyond herbivory, incorporating pollen limitation, water stress, edaphic factors, and other environmental aspects of each site.

To evaluate predictive models, we first looked for any predicted fitness records outside the bounds of fitness values actually recorded for each site; there was one, which was removed from downstream analyses. We then calculated the difference between the predicted fitness value and the observed fitness value for each record in each simulation (for plants that weren't eaten), and averaging across the 100 simulations, generating an "average difference" between simulation and reality for each plant. Looking at these differences across years, sites, and subspecies (Table B2), they are consistently small (except for *xantiana* at Center site in Year 1). This large difference between predicted

and observed values for *xantiana* at Center site in Year 1 is due to the model underpredicting fitness for several plants with extremely high observed fitness values.

Table B2. Average difference in observed and predicted seed set, averaged across 100 simulations, for each Year x Site x Subspecies combination.

Year	Site	Ssp	Mean seed set difference (Observed - predicted)
Wet	Center	p	9.1
Wet	Center	x	297.9
Wet	Edge	p	-4.7
Wet	Edge	x	4.5
Wet	Beyond	p	-10.5
Wet	Beyond	x	2.4
Dry	Center	p	-4.1
Dry	Center	x	-1.3
Dry	Edge	p	-1.0
Dry	Edge	x	-0.7
Dry	Beyond	p	-2.0
Dry	Beyond	x	-1.6

To see to what degree our simulations were changing the number of eaten plants with lifetime fitness above zero, we also calculated, for each simulation, the proportion of eaten plants that "retained" their observed fitness (zero) (Table B3). This would happen if the models predicted that even if that plant was not eaten, it would not have made any seed. These metrics generally conform to our expectations — roughly half of the *parviflora* and *xantiana* at the Edge site were predicted to make seed had they not been eaten (52% and 59%, respectively), but in the Beyond site in *parviflora* territory, even had *xantiana* not been eaten, only 39% of those plants were predicted to make seed, compared to 79% of *parviflora* (which did very well at the site). Only 27% of eaten *xantiana* were predicted to make seed at the Center site in Year 1, due to the fact that, even though fruiting *xantiana* made more seed in that site than others, the probability of a plant setting any seed in that site is actually quite low (mean probability of reproducing for *xantiana* in Center site in Year 1 = 0.22). In Year 2, survival was so low everywhere that most plants still died even when simulating no herbivory.

Table B3. Proportion of eaten plants that "retained" their observed fitness (zero), averaged across the 100 simulations.

Year	Site	Ssp	Percent of eaten plants where fitness remained zero
Wet	Center	p	0.87
Wet	Center	x	0.73
Wet	Edge	p	0.48
Wet	Edge	x	0.41
Wet	Beyond	p	0.21
Wet	Beyond	x	0.61
Dry	Center	p	0.92
Dry	Center	x	0.83
Dry	Edge	p	0.71
Dry	Beyond	p	0.86
Dry	Beyond	x	0.98

We also tested whether observed (from original field data) and simulated (for eaten plants; using a haphazardly chosen simulation replicate) non-zero fitness values at each site were drawn from significantly different distributions using Kolmogorov-Smirnov tests (Fig. S6). There was no indication that observed and simulated fitness values were drawn from different distributions at the Center (K-S = 0.23, P = 0.26) or Beyond-Edge (K-S = 0.10, P = 0.80) sites, but there was a significant difference in the underlying distributions of these values at the Edge site (K-S = 0.22, P = 0.01). This difference was likely due to the fact that non-zero simulated values for eaten plants in this simulation were slightly higher than observed values for plants that made seed in the field (4.33 vs 3.96; log scale). However, two observations lead us to be confident that our simulations are not overpredicting seed set for plants at the Edge site. First, when we average over all 100 simulations, there is trend toward *under*predicting seed set at this site (Table B2), Second, when we include eaten records where the model predicted fitness would equal zero, simulated values for eaten plants are lower than observed values for plants that made seed in the field (2.23 vs 3.96; log scale)

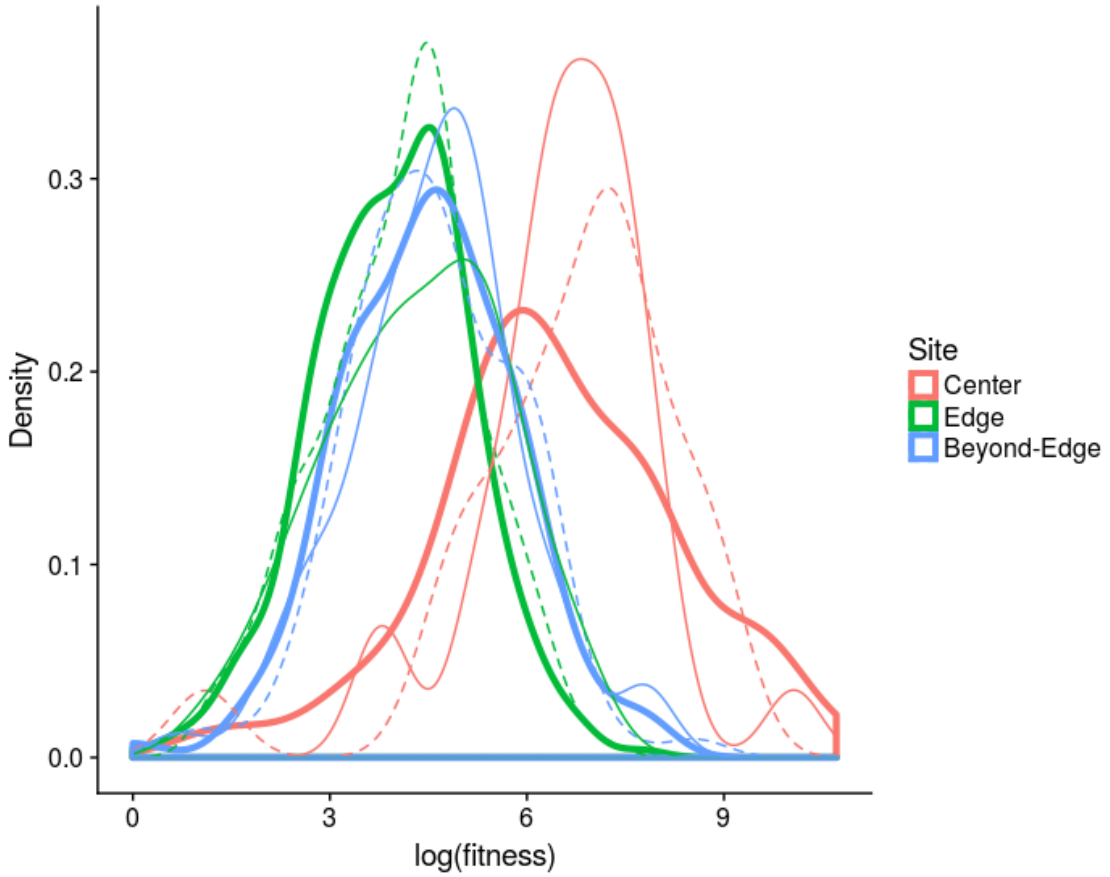

Figure B2. Distributions of fitness values for uneaten observed (plants that made seed in the field experiment; thick solid lines), uneaten simulated (using a haphazardly chosen simulation replicate; dashed lines), and eaten simulated (plants that were eaten but which, in the haphazardly chosen simulation replicate, were predicted to make seed; thin solid lines) plants, at each site in the transplant experiment. One eaten simulated *parviflora* record was removed from Site A (in this figure and in fitness analyses) because its predicted fitness values was above that of the maximum observed fitness value at that site.

To estimate average lifetime fitness we built linear mixed effects models as described in Methods (main text) for each of the 100 simulated data sets. Lifetime fitness estimates were averaged over the 100 simulations (Table B4). Comparison of the predicted model means using the original data and this no herbivory simulation estimates the effect of herbivory on population mean fitness for each subspecies at each site.

B.3: Simulation of fitness beyond the range edge with reduced herbivory

In the transplant experiment, herbivory rates beyond the range edge were ca. 100% higher than within the range (Results). Thus, we were also interested in simulating a more "moderate" scenario where herbivory was not completely absent, but rather, herbivory rates beyond the range edge were similar to rates within the range. In the wet year, 17% of all germinated plants were eaten at the Edge site, while 31% were eaten at the Beyond-Edge site (Table S1). In the dry year, these rates were 1 and 3%, respectively. We started with the 100 same simulated data sets described above, with eaten plants assigned predicted fitness values based on the performance of uneaten plants. Then, for each of the 100 simulations, we randomly chose individuals from the original set of eaten plants in the Beyond-Edge site to be "eaten" in the simulation (with the total number of eaten plants in each simulation reflecting the Edge site's herbivory rate for that year). To estimate average lifetime fitness we built linear mixed effects models as described in Methods (main text) for each of the 100 simulated data sets. The lifetime fitness estimates for each subspecies in the Beyond-Edge site for both years were averaged over the 100 simulations (Table B4). Comparison of the predicted model means using the original data and this reduced herbivory simulation estimates the effect of increased herbivory outside the range limit on *xantiana* population persistence.

B.4: Analyzing the subset of uneaten plants

We also analyzed the observed data after removing records of eaten plants (Table B4). This gives an additional comparison of fitness estimates for a scenario where the plants that were eaten simply never were planted. As we would expect, the estimates based on the subset of uneaten plants are higher than when using the full data set in the Wet year where herbivory was frequent, but sometimes not as high as when we simulate fitness values for those eaten plants (e.g., *xantiana* and *parviflora* in the Beyond-Edge site, and *xantiana* in the Edge site). The subset and simulated estimates will only be equal when the average simulated fitness of eaten plants is equal to the overall average fitness of the uneaten subset (which includes all planted seeds, including those that never germinated).

We can illustrate this by picturing a population of 40 planted seeds, where 4 seeds grew and each produced 100 seeds (fitness = 100), 10 plants grew but were eaten (fitness = 0), and 26 plants did not germinate. Averaging over the full data set, average fitness would be 10 seeds. Subsetting for only those plants that weren't eaten, average fitness would be 13.3 (400 total seeds / 30 plants). If we simulated "no herbivory" and 5 of the 10 eaten plants were predicted to make 100 seeds each (with the other eaten plants keeping fitness = 0), average fitness across all 40 plants would be 22.5.

Table B4. Average lifetime fitness estimates for each subspecies at each site in each year under four scenarios: "Observed", original field data from the reciprocal transplant; "Observed (no eaten plants)," which analyzed original field data after removing records of plants that were eaten; "No Herbivory" simulations, where we simulated fitness values for all plants eaten during the field experiment as if they hadn't been eaten; "Reduced herbivory Beyond-Edge", where we simulated lowered herbivory rates outside *xantiana*'s range limit (mimicking herbivory rates at the Edge site) but used the original data for Center and Edge sites. Standard errors of estimates in the No Herbivory and Reduced Herbivory simulations are averages from results of the 100 simulations.

Year	Site	Ssp.	**Average lifetime fitness** (± SE)			
			Observed	**Observed (no eaten plants)**	**No herbivory simulation**	**Reduced herbivory Beyond-Edge simulation**
Wet	Center	*parviflora*	0.34 ±0.25	0.54 ±0.33	0.49 ±0.30	0.34 ±0.25
Wet	Center	*xantiana*	2.50 ±0.71	3.37 ±0.99	3.50 ±0.99	2.5 ±0.71
Wet	Edge	*parviflora*	4.71 ±1.08	5.63 ±1.40	5.85 ±1.37	4.72 ±1.07
Wet	Edge	*xantiana*	2.70 ±0.75	5.83 ±1.60	7.87 ±1.95	2.70 ±0.75
Wet	Beyond-Edge	*parviflora*	2.38 ±0.65	3.08 ±0.87	4.95 ±1.21	3.63 ±0.88
Wet	Beyond-Edge	*xantiana*	0.15 ±0.24	0.28 ±0.31	1.07 ±0.47	0.60 ±0.33
Dry	Center	*parviflora*	0.01 ±0.19	0.01 ±0.21	0.03 ±0.21	0.01 ±0.19
Dry	Center	*xantiana*	0.02 ±0.21	0.03 ±0.23	0.03 ±0.23	0.02 ±0.21
Dry	Edge	*parviflora*	0.16 ±0.22	0.16 ±0.24	0.16 ±0.23	0.16 ±0.22
Dry	Edge	*xantiana*	0.03 ±0.21	0.03 ±0.23	0.03 ±0.23	0.03 ±0.21
Dry	Beyond-Edge	*parviflora*	0.09 ±0.21	0.09 ±0.23	0.09 ±0.22	0.09 ±0.20
Dry	Beyond-Edge	*xantiana*	0.03 ±0.21	0.03 ±0.23	0.03 ±0.23	0.03 ±0.21

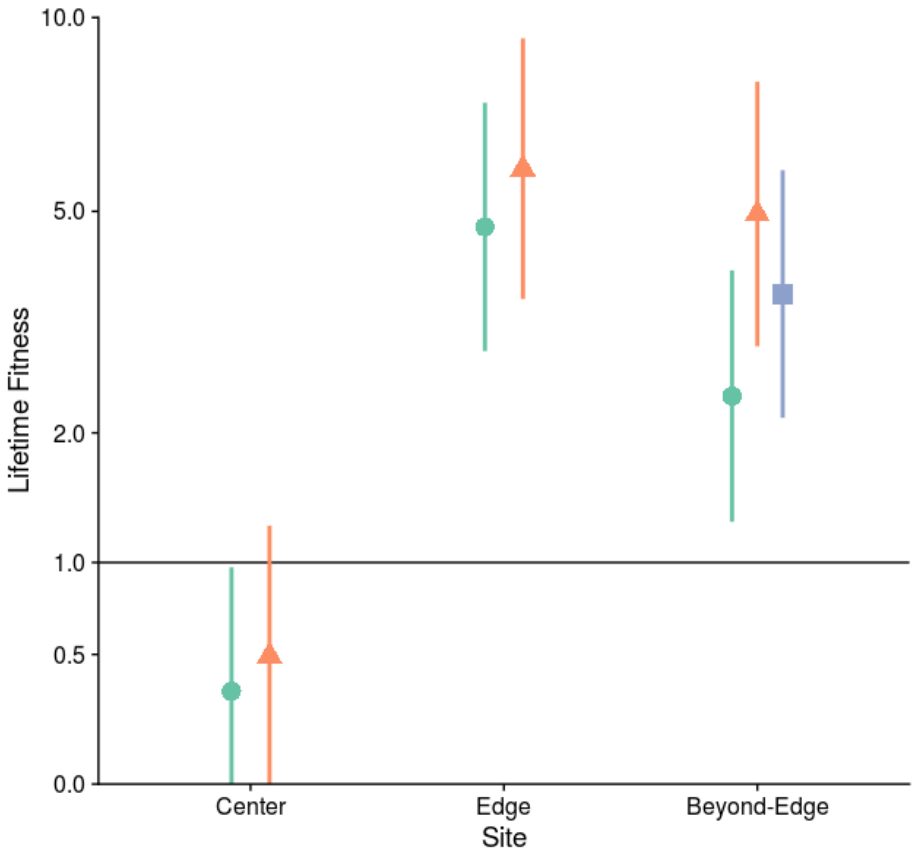

Figure B3. Lifetime fitness estimates for *parviflora* in each site in the Wet Year under observed (green circles) and two simulated scenarios: "No Herbivory" simulation (orange triangles), where we predicted fitness values for all plants eaten during the field experiment as if they hadn't been eaten; and "Reduced herbivory Beyond-Edge" (purple square), where we simulated lowered herbivory rates in the Beyond-Edge site (mimicking herbivory rates at the Edge site) but used the observed data for Center and Edge sites. Point ranges show 95% confidence intervals. Note Y axis is on log scale. Confidence intervals for simulation estimates are averages from results of 100 simulations.

C: Influence of phenology on probability of herbivory

C.1: Predicting flowering date for plants that died before flowering (only in wet year)

Since date of flowering can only be recorded on plants that flower before death, any plants that were eaten before they flowered would be excluded from analyses including flowering date as an independent variable. Thus, we predicted date of flowering for plants that died before flowering, enabling us to "recover" this missing phenological information and make more robust estimates of model parameters. To predict flowering date (days since 1 December, as a proxy for phenology) for plants that died (from herbivory or other factors) before flowering in the wet year, we used linear regression to model the effects of site, size in March, population, and all interactions on flowering date, using records of both species for which flowering date was recorded. This model explained variation in flowering date very well ($R^2 = 0.86$), and we used the model to predict flowering date for all records that did not have a flowering date recorded in the field.

To evaluate predictive models, we first calculated the mean difference between observed and predicted flowering date for all individuals with flowering date recorded in the field; the mean difference was 3.5 days. We also checked to see if any predicted flowering dates fell outside the range of observed flowering dates at any site; all predicted flowering dates fell within the observed range. We then compared observed vs predicted flowering date for all records where flowering date was recorded in the field; the resulting plot showed no signs of consistent bias (Fig. S8).

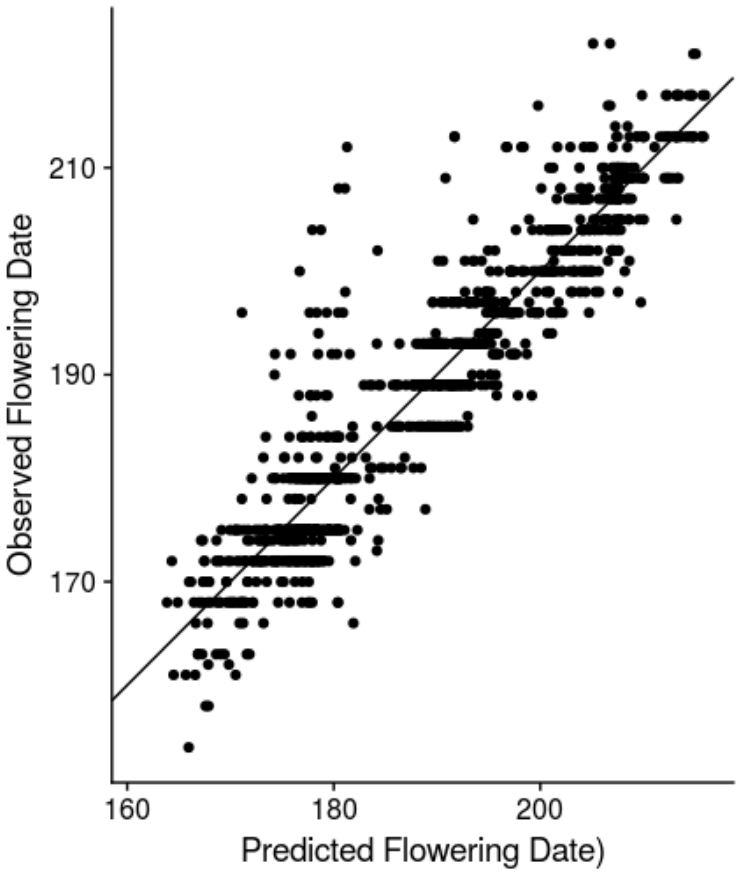

Figure C1. Plot of observed flowering date of plants vs. flowering date as predicted by the model used to predict flowering date for plants that died before flowering. Identity line plotted for reference, where y = x.

C.2: Using predicted flowering dates

Results in the main text report on logistic regressions of herbivory on phenology using these predicted flowering dates. For comparison, we also ran these analyses (described in Methods) with the dataset containing only plants whose flowering date was recorded in the field (i.e. excluding plants that died prior to flowering). Results for the Beyond-Edge site were qualitatively similar to those using predicted flowering date, though with higher estimates for the effect of phenology (observed records only: 0.19 vs. including predicted records: 0.13). Phenology explained no significant variation in odds of herbivory at the Center or Edge site when modeled with observed flowering dates only.

C.3: Checking for confounding of subspecies and phenology

Given the phenological differentiation between *xantiana* and *parviflora*, it is possible that the difference in probability of herbivory due to plant phenology was in part confounded with some other difference between the subspecies. To test if the influence of phenology on herbivory was reflecting some other inherent difference between *xantiana* and *parviflora*, we added subspecies as a term to our analyses reported in the main text that used predicted flowering dates. Thus we tested the effect of date of flowering, with plant size, block, and subspecies as covariates, on a plant's probability of fatal herbivory at the three sites using logistic regression. Despite the collinearity of subspecies and flowering date, flowering date was still highly significant beyond the range edge, but was not a significant predictor at the Center or Edge sites (Table S4).

With collinear model terms, standard errors of the affected coefficients increase (Graham 2003). When testing significance of model terms, this can lead to a Type II error (i.e., failing to reject a false null hypothesis of no effect of a predictor variable). Given the relatively weaker effect of flowering date on herbivory at the Center and Edge sites (Table S6), this is likely why flowering date was not a significant predictor when including subspecies as a model term. However, where flowering date influences herbivory most strongly (Beyond-Edge), LR tests still identified flowering date as a strong predictor. Thus we believe our findings of phenology's effects on probability of herbivory are not confounded with some other aspect of subspecies differentiation.

Table C1. Results of Type II Analysis of Deviance using likelihood ratio tests for the logistic regression model, Herbivory ~ Phenology + Subspecies + Size + Block, for both *xantiana* and *parviflora* in Center, Edge, and Beyond-Edge sites in the wet year.

		Center		**Edge**		**Beyond-Edge**	
	df	χ^2	Estimate	χ^2	Estimate	χ^2	Estimate
Date of flowering	1	0.5	-0.01	2.7	0.03	14.24***	0.10
Subspecies	1	2.3	--	3.7	--	0.8	--
Size	1	41.0***	-0.001	16.7***	.002	9.9**	0.001
Block	9	117.3***	--	80.0***	--	81.9***	--

*, **, and *** indicate P values lower than 0.05, 0.01, and 0.001, respectively.

C.4: Including early season herbivory in models of herbivory ~ phenology

Field observations and temporal trends in herbivory during the transplant experiment (Fig. S4) suggested that subspecies' differences in herbivory become especially pronounced in the latter half of the growing season (April - June), but there was also some herbivory during the early growing season. To see whether our results would change if we included this early season herbivory, we analyzed the effects of phenology on herbivory as in the main text, but included herbivory events that happened prior to the March census. Results (Table S5) are qualitatively similar to those reported for late season herbivory only (Table S6), with phenology strongly influencing probability of herbivory at the range edge and beyond.

Table C2. Results of Type II Analysis of Deviance using likelihood ratio tests for the logistic regression model, Herbivory ~ Phenology + Size + Block, for both *xantiana* and *parviflora* in Center, Edge, and Beyond-Edge sites in the wet year. These models included plants that were eaten during early season herbivory.

		Center		**Edge**		**Beyond-Edge**	
	df	χ^2	Estimate	χ^2	Estimate	χ^2	Estimate
Date of flowering	1	2.2	0.01	71.1***	.06	114.5***	0.09
Size	1	30.6***	-0.001	28.6***	.003	0.14	<0.001
Block	9	114.5***	--	84.7***	--	61.7***	--
				————————			
Nagelkerke's Pseudo R^2		0.23		0.36		0.65	

*, **, and *** indicate P values lower than 0.05, 0.01, and 0.001, respectively.

Table C3. Results of Type II Analysis of Deviance using likelihood ratio tests for the logistic regression model, Herbivory ~ Phenology + Size + Block, for both *xantiana* and *parviflora* in Center, Edge, and Beyond-Edge sites in the wet year.

		Center		**Edge**		**Beyond-Edge**	
	df	χ^2	Estimate	χ^2	Estimate	χ^2	Estimate
Date of flowering	1	3.9*	0.02	53.8***	.05	118.0***	0.13
Size	1	39.0***	-0.001	18.0***	.002	9.8**	0.001
Block	9	116.1***	--	78.4***	--	82.0***	--
Nagelkerke's Pseudo R^2		0.63		0.47		0.87	

*, **, and *** indicate P values lower than 0.05, 0.01, and 0.001, respectively.

C.5: Effects of phenology on herbivory for *xantiana* only

We also tested the effect of date of flowering, with plant size and block as covariates, on a plant's probability of fatal herbivory at each site using logistic regression for *xantiana* alone (not including *parviflora*). When we analyzed *xantiana* alone, phenology only influenced probability of herbivory at the Edge site, where each day of delayed flowering increased *xantiana*'s odds of herbivory five percent ($P = 0.03$) (Table S7). There was no influence of phenology on herbivory at the Beyond-Edge site, likely because for *xantiana*, the overall later flowering at this site combined with the subspecies' delayed flowering caused almost the whole range of *xantiana* phenology phenotypes to be exposed to late season herbivory. The relationship of herbivory with size remained the same as in the cross-subspecies analysis, with larger plants more likely to be eaten in the Edge and Beyond Edge sites, but less likely to be eaten at the Center site ($P < 0.01$ for all sites).

Table C4. Results of Type II Analysis of Deviance using likelihood ratio tests for the logistic regression model, Herbivory ~ Phenology + Size + Block, for *xantiana* in Center, Edge, and Beyond-Edge sites in the wet year.

		Center		**Edge**		**Beyond-Edge**	
	df	χ^2	Estimate	χ^2	Estimate	χ^2	Estimate
Date of flowering	1	3.2	-0.06	4.7*	0.05	0.4	0.03
Size	1	30.1***	-0.001	8.0**	0.001	10.7**	0.001
Block	9	36.2***	--	67.8***	--	47.4***	--
Nagelkerke's Pseudo R^2		0.66		0.44		0.88	

*, **, and *** indicate P values lower than 0.05, 0.01, and 0.001, respectively.

C.6: Selection differentials for phenology

As another way of investigating the relationship between plant phenology and herbivory, we calculated selection differentials for phenology, comparing mean flowering date for three groups at each site in the wet year:

1. Base population (all plants alive in March)
2. Reproductive or eaten population (plants that were either eaten or produced seed)
3. Reproductive population (plants that produced seed)

From these groups we calculated:

1. Overall selection differential (**Overall S**)
    a. Equal to: mean flowering date of group 3 - mean flowering date of group 1
2. Selection differential due to factors other than herbivory (**No-herbivory S**)
    a. Equal to: mean flowering date of group 2 - mean flowering date of group 1

The difference between **Overall S** and **No-herbivory S** indicates the selection differential due to herbivory, **Herbivory S** (also equal to mean flowering of group 3 - mean flowering date of group 2).

We calculated these metrics for the subspecies alone, and also lumping them both together (which gives a bimodal distribution of flowering time, and is not a proper selection differential, but better reflects the influence of phenological differences between the ssp). Selection for earlier flowering due to herbivore pressure was apparent for both subspecies at the Edge and Beyond Edge sites (Table S8), though more pronounced in *xantiana*.

Table C5. Selection differential for flowering date due to herbivory (**Herbivory S**) for both subspecies alone, and grouped together, at each transplant site during the wet year.

	xantiana	***parviflora***	***Both ssp.***
Center	0.26	0.97	5.73
Edge	-0.76	-0.02	-3.13
Beyond-Edge	-1.25	-0.68	-4.56

* negative S means selection for earlier flowering

C.7: Estimating optimal flowering date across *xantiana*'s range

Our stem translocation experiments mapped the gradient in probability of herbivory across and beyond *xantiana*'s range. In theoretical explorations of range limits phenomena, these environmental gradients are tied to clines in organismal trait optima. Though we cannot estimate trait optima in phenology at as fine a scale as we did probability of herbivory, we can estimate optimal phenology at each transplant site by looking at the relationship between flowering date and fitness.

We estimated optimal flowering date by fitting a loess smoother to the function *log(fitness) ~ flowering date* (loess function in R with span = 1). We included both subspecies to increase the phenological range over which we could evaluate fitness, and included all plants that were alive at the March census. We only included records with flowering dates falling between the 2.5 and 97.5 percentiles of flowering date at each site to avoid phenological extremes.

At the Center and Beyond-Edge sites there were clear flowering dates at which fitness was maximized, but the optimal flowering date at the Edge site was less clear; the loess function remains relatively flat from the earliest flowering date until the beginning of *xantiana*'s flowering range (Figure S8). Thus, to estimate a conservative optimal flowering date at the Edge site, we calculated the earliest flowering date at which the loess fitness prediction dropped below the lower 95% CI of the highest fitness prediction. Since there were site effects of phenology (with the Beyond-Edge site flowering later overall relative to Center and Edge), we standardized this optimal flowering date by comparing it to the mean *xantiana* flowering date for each site.

At the Center and Edge sites, the mean *xantiana* flowering date was within four days of the optimal flowering date. At the Beyond-Edge site, the optimal flowering date was 18 days before the mean *xantiana* flowering date.

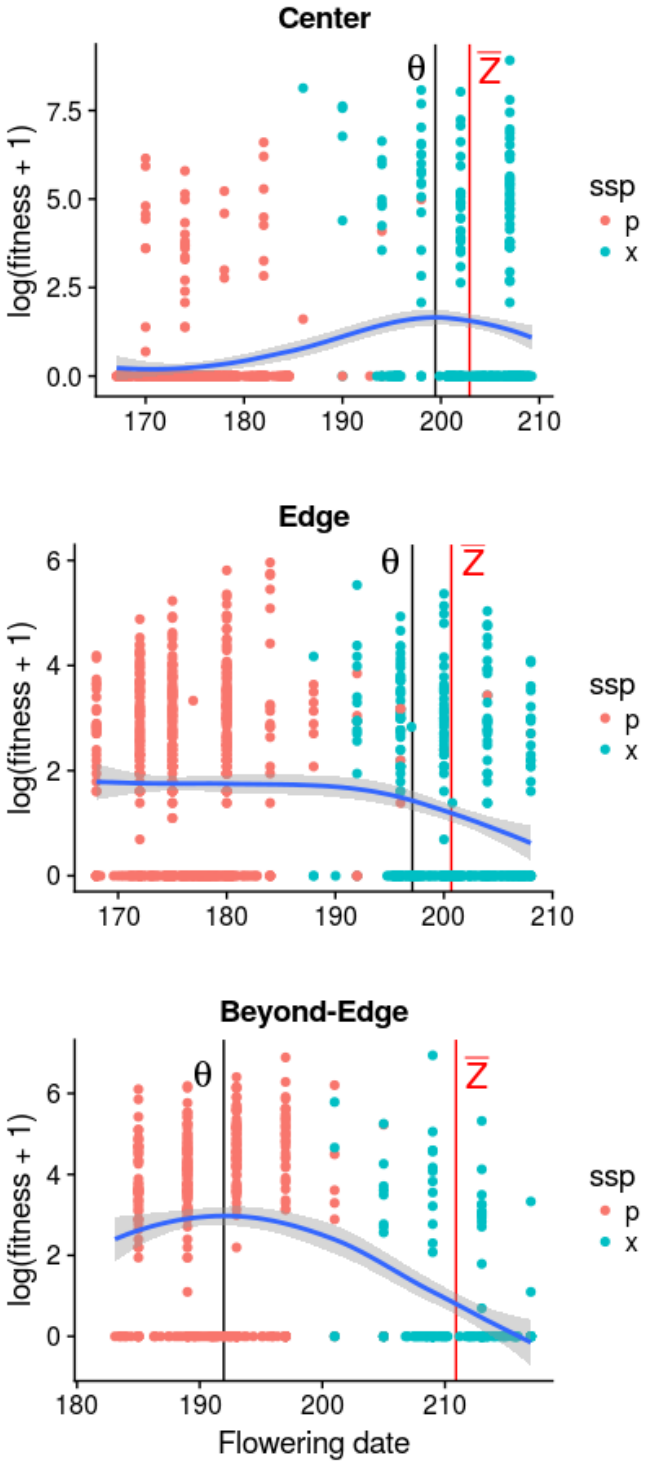

Figure C2. We used a loess smoothing function to compare the optimal flowering date where fitness was maximized (marked by the black line labeled θ) to the mean *xantiana* flowering date (marked by the red line labeled $\overline{Z}$) at each site during the wet year. See text for details of estimation for Edge site.

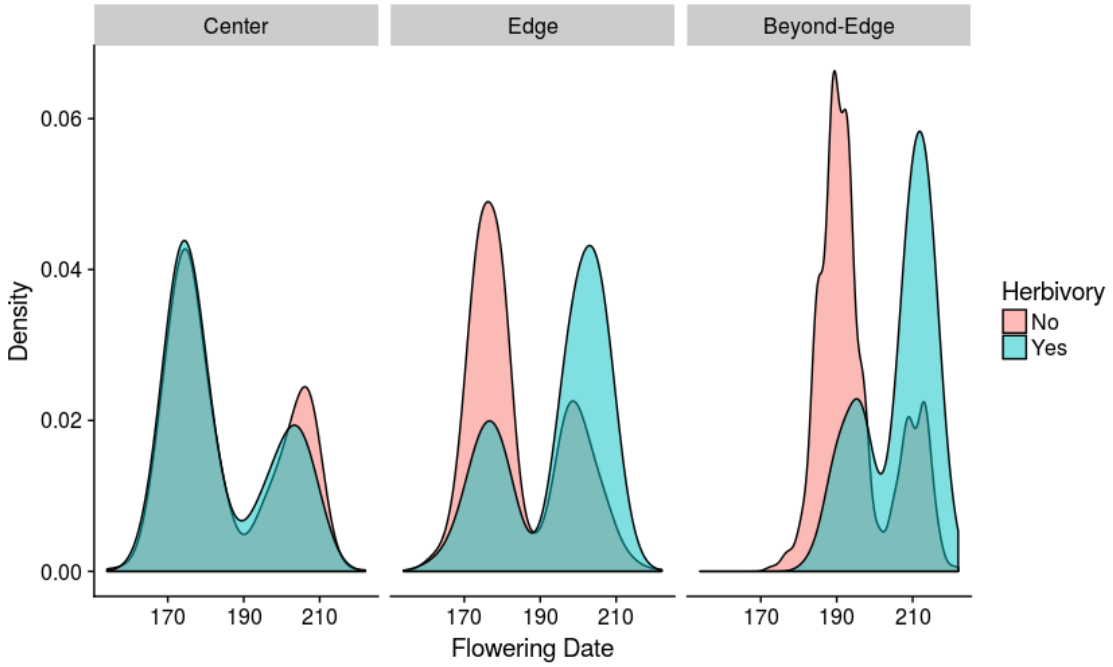

Figure C3. Kernel density estimates (i.e., smoothed histograms) of phenology for eaten (blue) and uneaten (pink) plants at each site. Bimodality of distributions reflects differentiation in phenology between the two subspecies.

References

Graham, M.H. (2003). Confronting Multicollinearity In Ecological Multiple Regression. *Ecology*, 84, 2809–2815.

Appendix 2

SI.1

Table S1. Site locations, growing season precipitation, and fatal herbivory during experiment years. Herbivory rates are calculated as the proportion of uncaged plants that were eaten, where the denominator equals the total number of plants that survived at least until the May census or were eaten between March and May.

Site Name	**Site Number*** **	**Latitude, Longitude**	**Cumulative Growing Season Precipitation (mm)**		**Percent fatal herbivory (eaten / total plants)**		**Density of *C. x. xantiana* (fruiting plants per ½ m^2)**** **
			Year 1	***Year 2***	***Year 1***	***Year 2***	
Center	109x	35.529298, -118.654624	446.0	615.4	0.02 (3 / 139)	0.05 (1 / 20)	0.63
Intermediate	57x	35.6240674, -118.515680	418.4	747.6	0.05 (4 / 77)	0.20 (13 / 65)	0.18
Edge	92x	35.679532, -118.474717*	383.8	803.4	0.20 (12 / 61)	0.04 (3 / 70)	0.23
Just Beyond	66p	35.676131, -118.355300	164.0	379.4	None (0 / 5)	0.36 (47 / 132)	*NA*
Beyond	68p	35.614754, -118.250653	167.2	*No data***	0.28 (11 / 39)	0.38 (70 / 182)	*NA*
Far Beyond	55p	35.7161517, -118.169986*	164.6	447.6	None (0 / 3)	0.12 (9 / 74)	*NA*

* Precipitation for Edge and Far Beyond sites gathered from nearby weather stations at (Lat: 35.645708, Long: -118.472720) and (Lat: 35.667818, Long: -118.055605), respectively.

** The weather station at the Beyond site was destroyed in a wildfire in late 2016. For that site in Fig. 2, we present data for year 2 from the nearby Just Beyond site.
*** Site numbers correspond to those used in our larger *C. xantiana* population database
**** Density of fruiting plants per ½ m^2 averaged over years 2013-2017

SI.2

Estimating seed number from fruit weight

We estimated seed number of individual fruits using a linear model with fruit weight as a predictor. We counted seeds in a subset of weighed fruits, and then split these fruits into model training (354 fruits) and testing (168 fruits) data sets. We built the model using seed counts from the training set, removing outliers based on Cook's distance. The model predicted seed number well ($R^2 = 0.75$). We then used the model to predict seed number of fruits in the testing set. The average difference between observed and predicted seed number was -0.58, and the SD of that difference was 9.32. We then used this model to predict seed number for all other fruits collected in the experiment.

SI.3

Testing for caging effects on plant growth

Cages and other structural exclosures can affect plant growth through shading, wind buffering, or altering microclimate temperatures. We tested for a caging effect on plant growth by examining average plant leaf number (a proxy for size) of caged and uncaged plants in the latter part of the growing season when cages had been in place for ca. two months (March - May). We used data from year 1 only to allow us to compare plant size between caging treatments without any confounding effects of herbivory (some herbivory in year 2 occurred before our first May census and could thus shift the distribution of plant size for uncaged plants). Average sizes of caged and uncaged plants are shown in Table S2. We used a linear model to test whether site, caging treatment, or their interaction influenced plant size (log transformed). Type II ANOVA indicated that whilst site was a strong predictor of plant size ($P < 0.0001$), there was no effect of caging treatment ($P = 0.54$) or a significant site $\times$ caging interaction ($P = 0.88$).

Table S2. Average plant size of caged and uncaged plants at each site in year 1 after ca. 2 months of caging.

Site	Average plant size (n)	
	Uncaged	**Caged**
Center	42.5 (133)	39.1 (120)
Intermediate	10.5 (74)	9.6 (70)
Edge	16.2 (60)	17.5 (66)
Just Beyond	5.5 (4)	5 (2)
Beyond	7.1 (37)	5.4 (44)
Far Beyond	4.7 (3)	4.0 (3)

SI.4

Effect of plant size on probability of herbivory

We tested whether plant size in March (number of leaves, measured at the Early Survival census) influenced probability of fatal herbivory on uncaged plants at the four sites with high herbivory in year 2 (Intermediate, Just Beyond, Beyond, Far Beyond). We used logistic regression with site, March size, and their interaction as predictors. A Type II ANOVA indicated that March size significantly influenced probability of herbivory (LR $\chi^2 = 77.5$; $P < 0.0001$), with each additional leaf increasing a plant's odds of herbivory 11%. Site and the site $\times$ size interaction were not significant.

SI.5

Effects of precipitation on lifetime fitness

We did not explicitly include precipitation in our main analyses due to two main issues: precipitation is perfectly confounded with site and strongly autocorrelated with range position (inside and outside), and there are only six precipitation values for each year of the experiment (one for each site), which is not ideal for treating this term as a continuous variable. However, below we offer a supplementary analysis of the isolated effects of precipitation on mean lifetime fitness during the two years of the experiment.

We used linear regression to estimate the effect of growing season precipitation on *xantiana* mean lifetime fitness within each site, analyzing each year separately and only including plants in the caged treatment to control for herbivory effects on fitness. In year 1, we log transformed mean lifetime fitness to meet assumptions of ANOVA; fitness

did not require transformation in year 2. We used ANOVA to test the significance of precipitation as a predictor of fitness.

In year 1, mean lifetime fitness increased with increasing precipitation (m = 0.02, P = 0.008; Fig. S1a), which reflects the stark contrast between beyond range sites (all < 170 mm precipitation and mean fitness near zero) and within range sites (all > 380 mm precipitation and mean fitness ranging from 0.9 – 34.6 seeds per planted seed). In year 2, when all sites received more than 350 mm rainfall, there was a trend for fitness to decrease with increasing precipitation, though the slope was not significantly different from zero (m = -0.008, P = 0.15; Fig. S1b); however, one should keep in mind that these regressions, based on only six data points, are power limited.

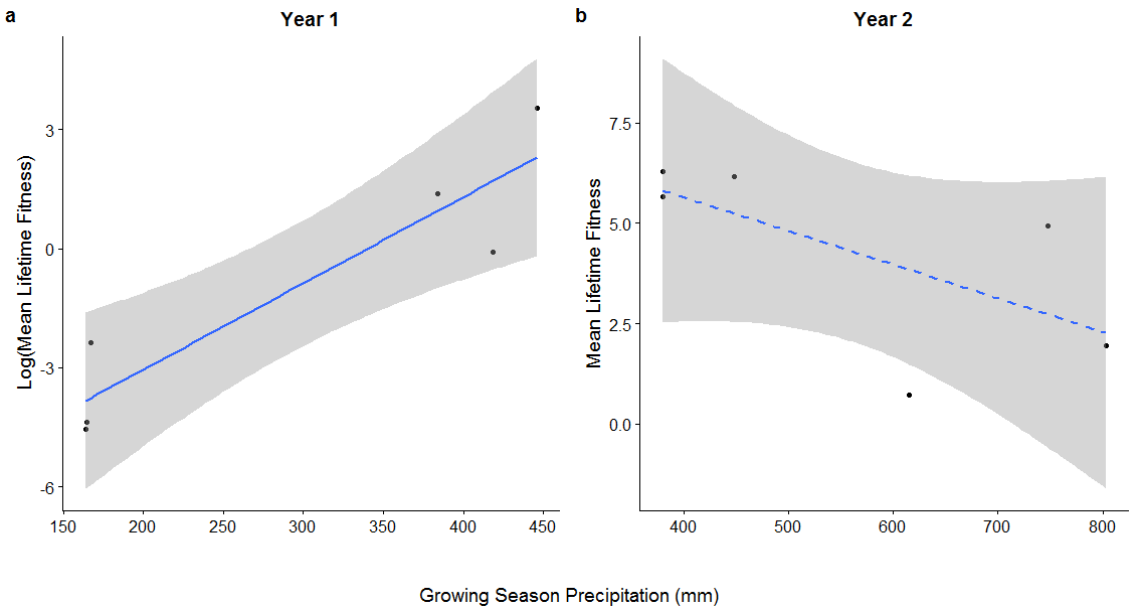

Figure S1. Mean lifetime fitness plotted against growing season precipitation for years 1 (a) and 2 (b); data points are site means of plants in the caged treatment. Linear regressions with 95% confidence bands are overlaid on both plots; slope is not significantly different from zero in plot b.

Appendix 3

Table S1. Summary of LRT contrasts comparing lifetime fitness estimates between inoculum sources in the field experiment. The first set of contrasts (at Center, Intermediate, and Edge sites) asks, for each source population, whether lifetime fitness differed between plants grown with control inoculum and those grown with their home site inoculum. Here, higher fitness with one's home inoculum indicates adaptation, while the reverse indicates maladaptation. The second set of contrasts (at Just Beyond, Beyond, and Far Beyond sites) asks, for each source population, whether the addition of any of the three soil inocula from within *xantiana*'s range improved lifetime fitness of plants when planted outside the range limit. Here, higher fitness with inoculum from inside the range indicates adaptation, while the reverse indicates maladaptation. Bold *P* values remain significant after Holm adjustment.

Site	Source Population	Inoculum Contrast	Result	Dev	P
Center	Intermediate	Intermediate vs. Control	Maladaptation	5.4	0.019†
	Edge	Edge vs. Control	NS	0.5	0.475
Intermediate	Center	Center vs. Control	Adaptation	8.1	**0.004**
	Edge	Edge vs. Control	NS	0.2	0.693
Edge	Center	Center vs. Control	Maladaptation	3.8	0.051
	Intermediate	Intermediate vs. Control	Adaptation	2.9	0.088
Just Beyond	Center	Center vs. Control	NS	0.04	0.835
		Intermediate vs. Control	Adaptation	8.7	0.003†
		Edge vs. Control	Maladaptation	4.7	0.029
	Intermediate	Center vs. Control	NS	0.7	0.399
		Intermediate vs. Control	NS	2.6	0.108
		Edge vs. Control	NS	1.9	0.169
	Edge	Center vs. Control	Maladaptation	4.5	0.034
		Intermediate vs. Control	NS	0.5	0.475
		Edge vs. Control	NS	0.5	0.471

Site	Source Population	Inoculum Contrast	Result	Dev	P
Beyond	Center	Center vs. Control	NS	0.1	0.702
		Intermediate vs. Control	Adaptation	8.1	0.004†
		Edge vs. Control	Adaptation	9.5	0.002†
	Intermediate	Center vs. Control	NS	0.1	0.725
		Intermediate vs. Control	NS	0.0	0.987
		Edge vs. Control	Maladaptation	11.8	**0.0006**
	Edge	Center vs. Control	NS	0.1	0.724
		Intermediate vs. Control	Maladaptation	5.7	0.017
		Edge vs. Control	Maladaptation	5.6	0.018
Far Beyond	Center	Center vs. Control	NS	0.7	0.387
		Intermediate vs. Control	Maladaptation	4.7	0.031
		Edge vs. Control	Maladaptation	5.6	0.018
	Intermediate	Center vs. Control	Adaptation	9.3	0.002†
		Intermediate vs. Control	NS	0.6	0.444
		Edge vs. Control	NS	1.8	0.183
	Edge	Center vs. Control	NS	3.1	0.08
		Intermediate vs. Control	NS	0.5	0.5
		Edge vs. Control	Adaptation	6.9	0.009

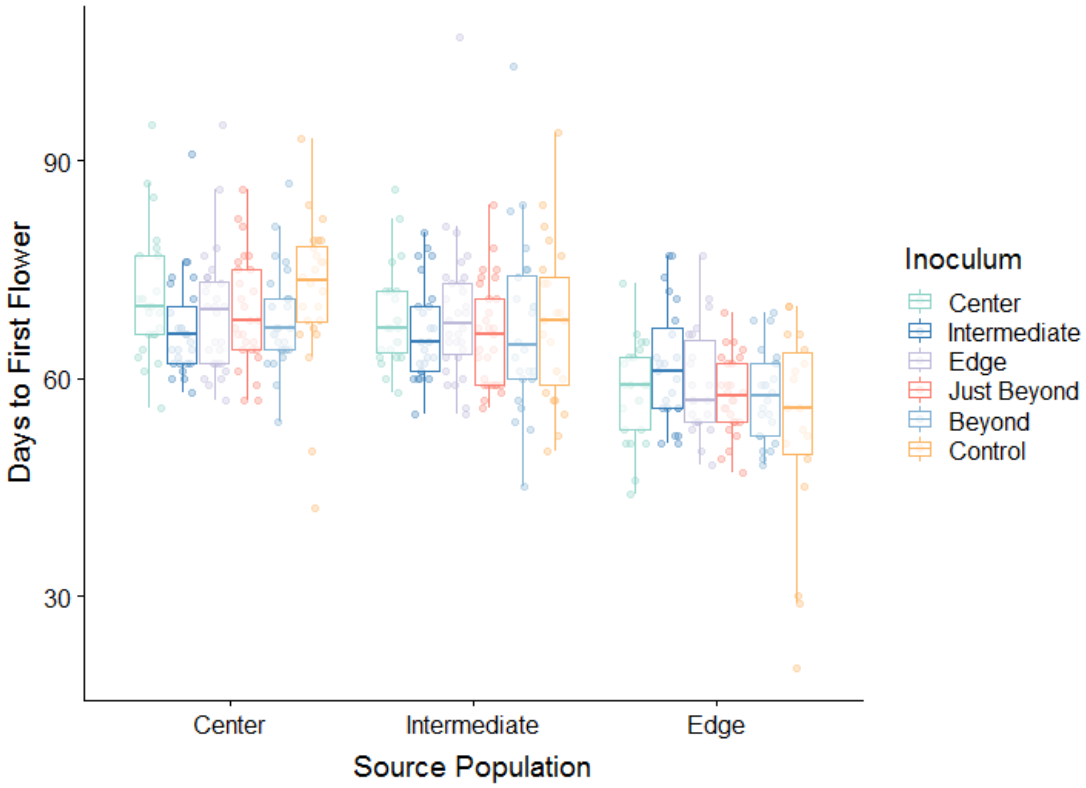

Figure S1. Effects of source population and inoculum source on flowering phenology in the greenhouse experiment.

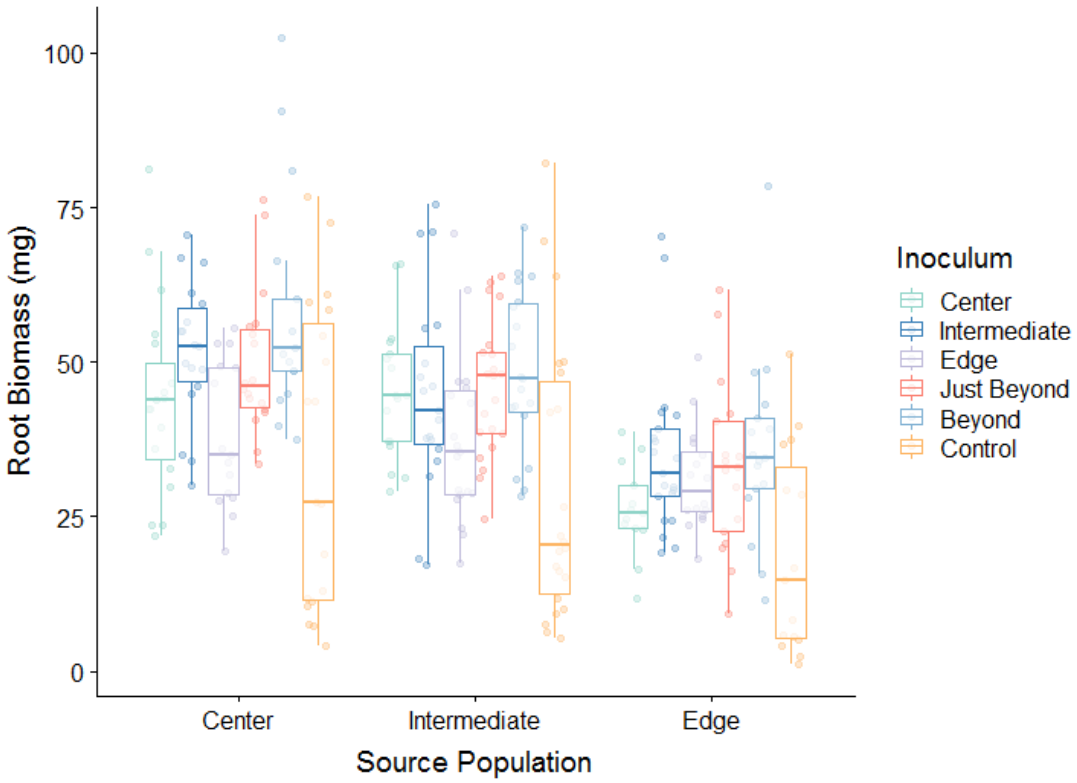

Figure S2. Effects of source population and inoculum source on root biomass in the greenhouse experiment.

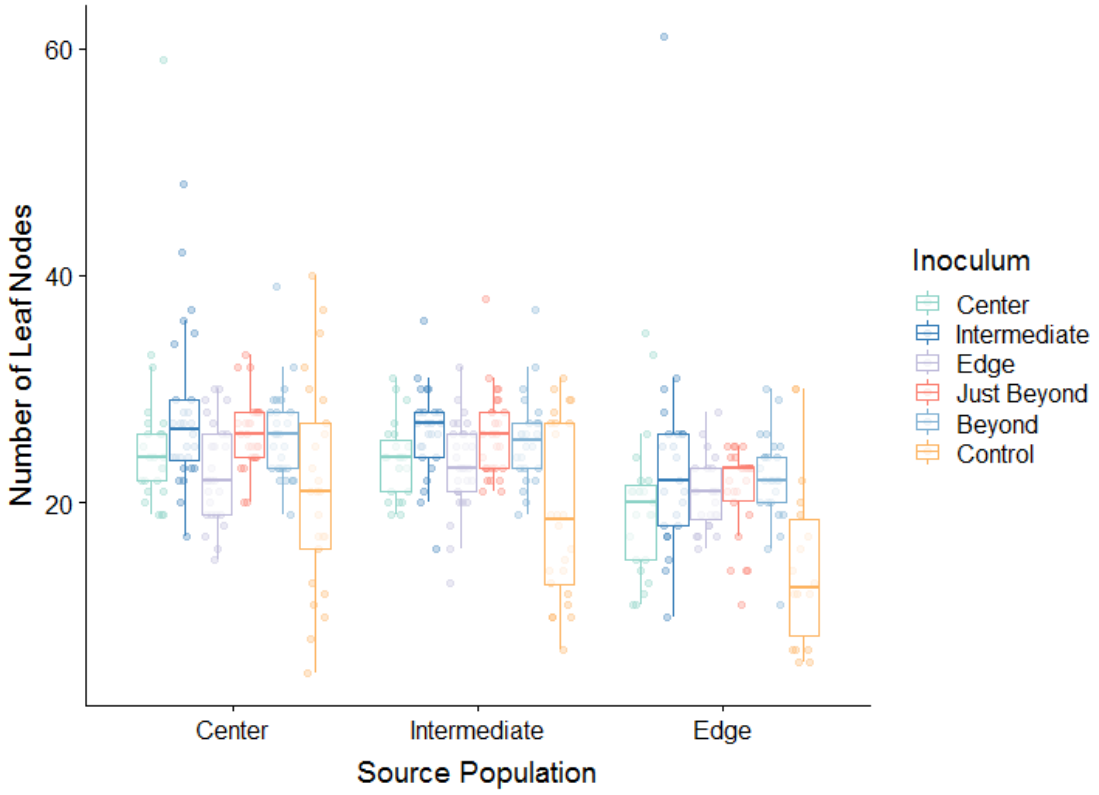

Figure S3. Effects of source population and inoculum source on number of leaf nodes in the greenhouse experiment.

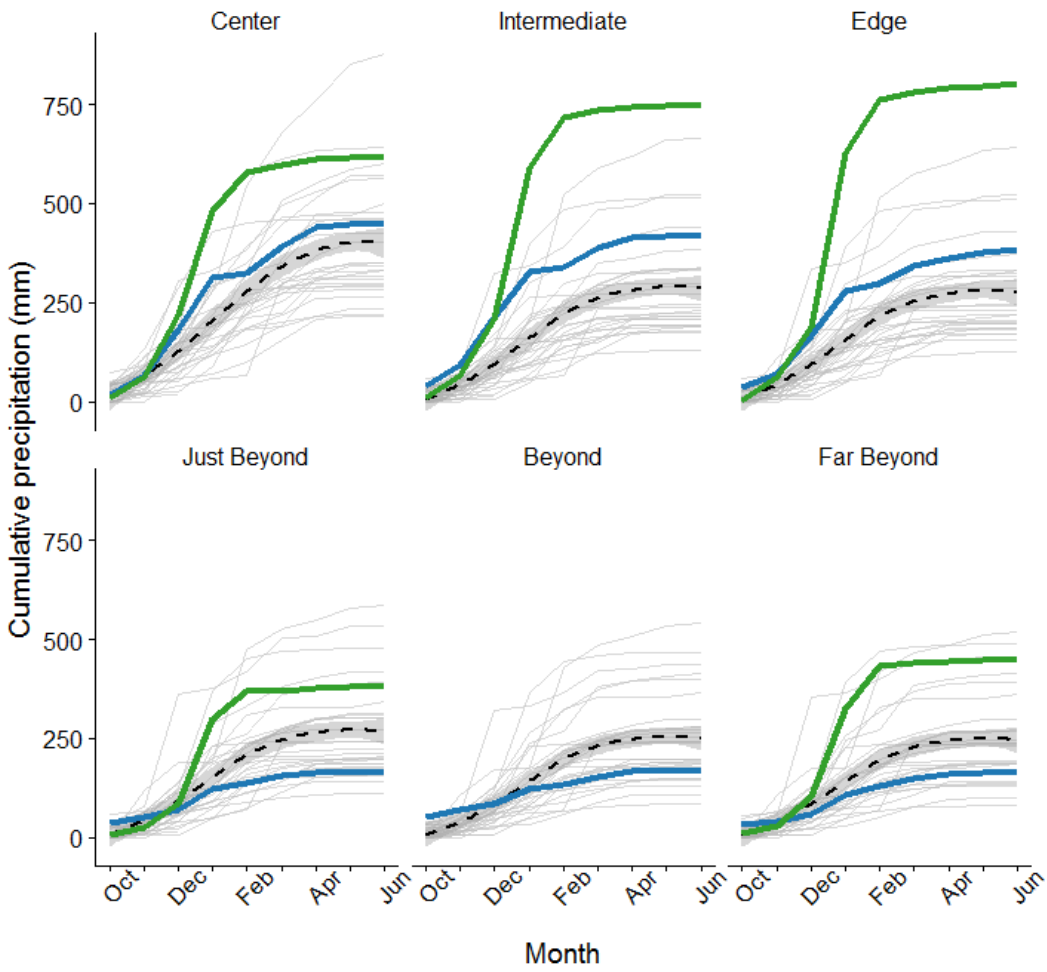

Figure S4. Cumulative precipitation across the growing season (October - June) in the field experiment. Shown are precipitation patterns during the transplant experiment (year 1: blue lines; year 2: green lines), using data from weather stations at or near the sites. We also plotted precipitation for the years 1990 - 2017 at each site location (thin grey lines), using interpolated estimates from PRISM, to help interpret study year precipitation patterns in the context of long term trends (dashed black line shows long term trend with 95% confidence band). Precipitation data for the Beyond site in year 2 was unavailable due to a wildfire destroying our weather station.

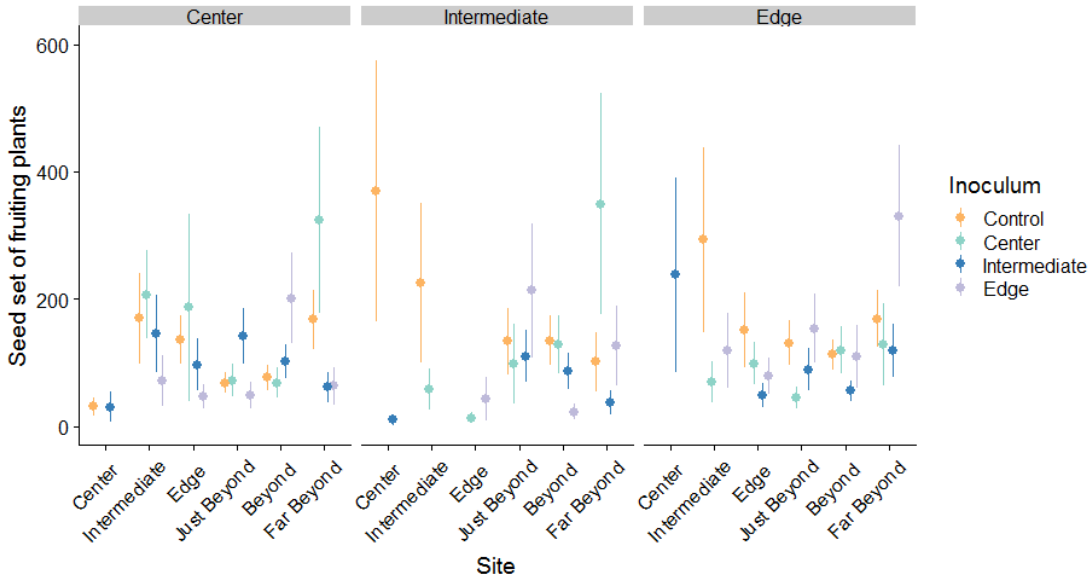

Figure S5. Effects of site and inoculum source on seed set of fruiting plants for each source population; source populations are displayed in separate panels. Estimates (± 95% CI) are estimated marginal means from the negative binomial regression of seed set on site, source population, inoculum, and their interactions.

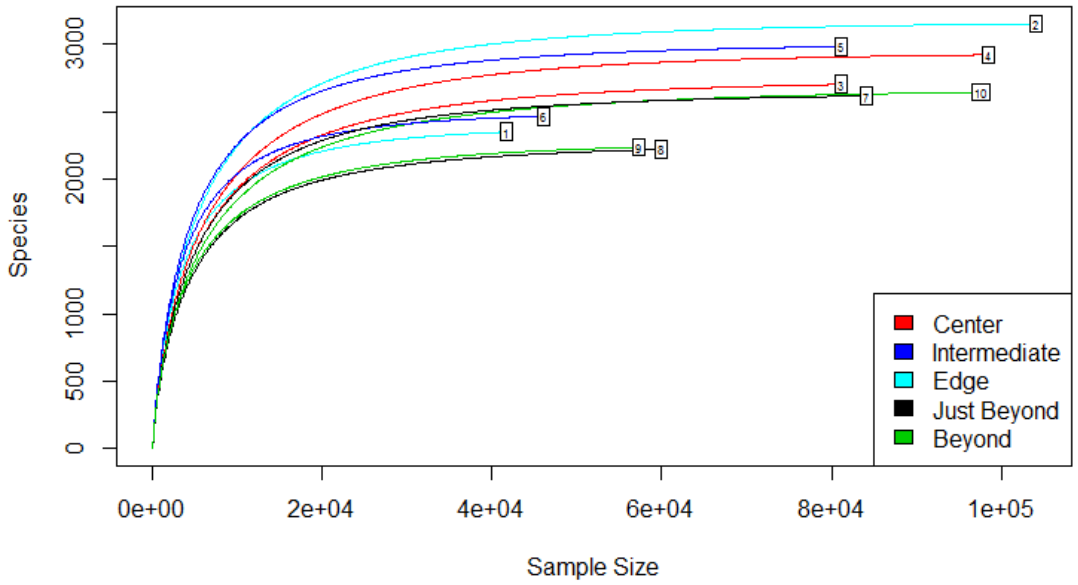

Figure S6. ASV accumulation curves for the 10 inoculum samples (two per inoculum source) from the greenhouse experiment.

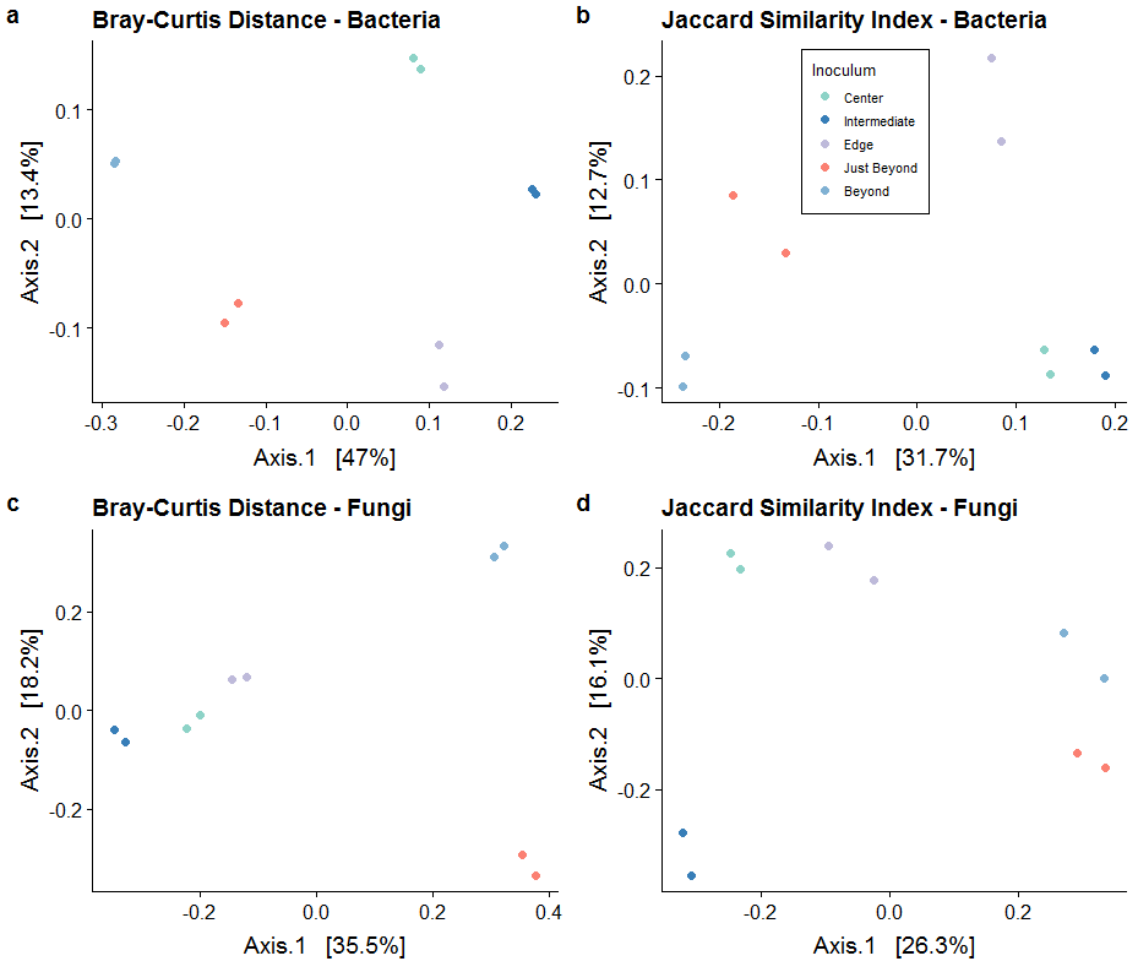

Figure S7. PCoA for Bray-Curtis distance **(a, c)** and Jaccard similarity index **(b, d)** matrices comparing bacterial **(a, b)** and fungal **(c, d)** community composition among inoculum sources from the greenhouse experiment.

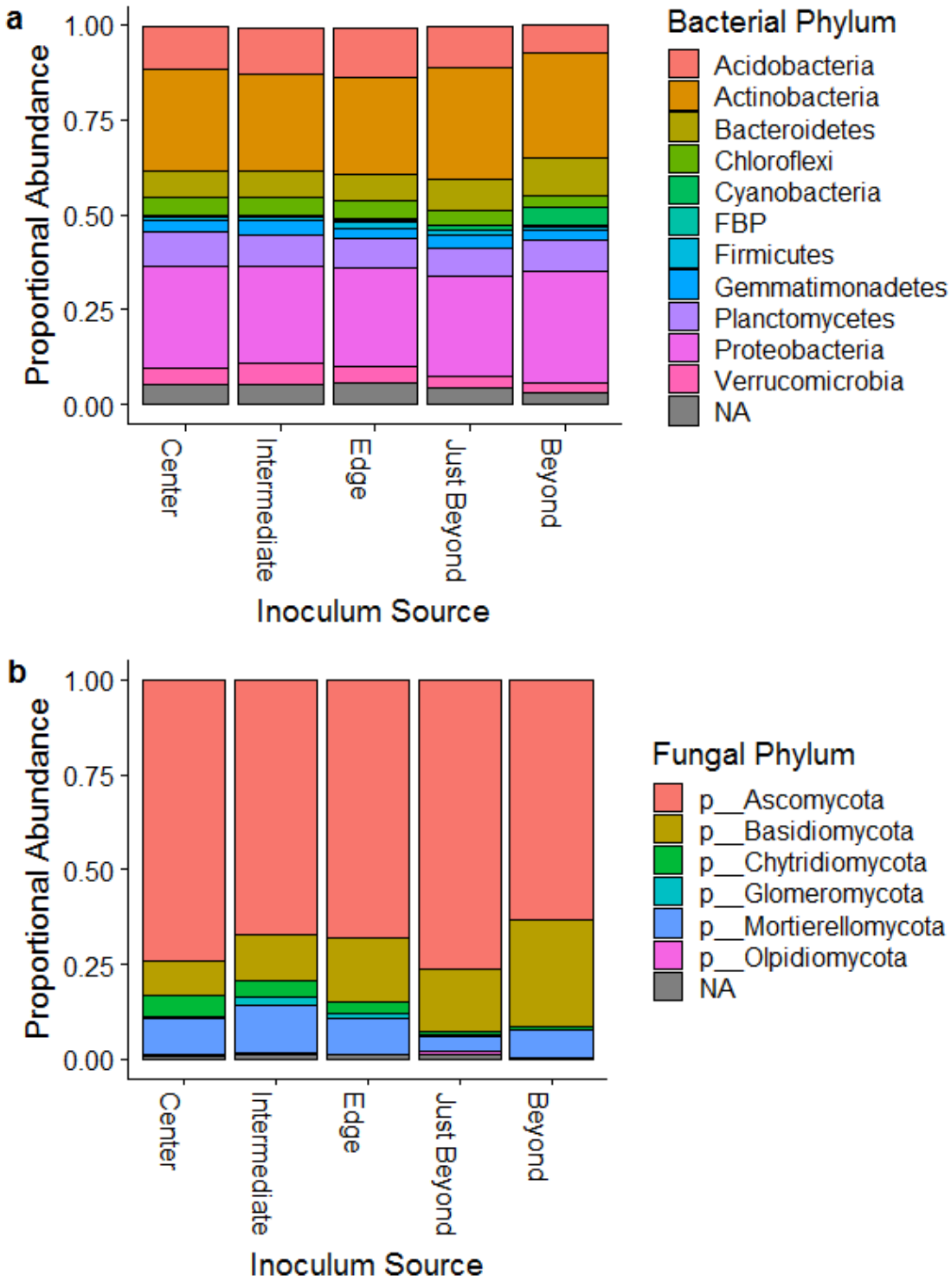

Figure S8. Proportional read abundance of **a)** bacterial and **b)** fungal phyla across inoculum sources from the greenhouse experiment. These plots include only those phyla whose proportional abundance across samples was > 0.01.

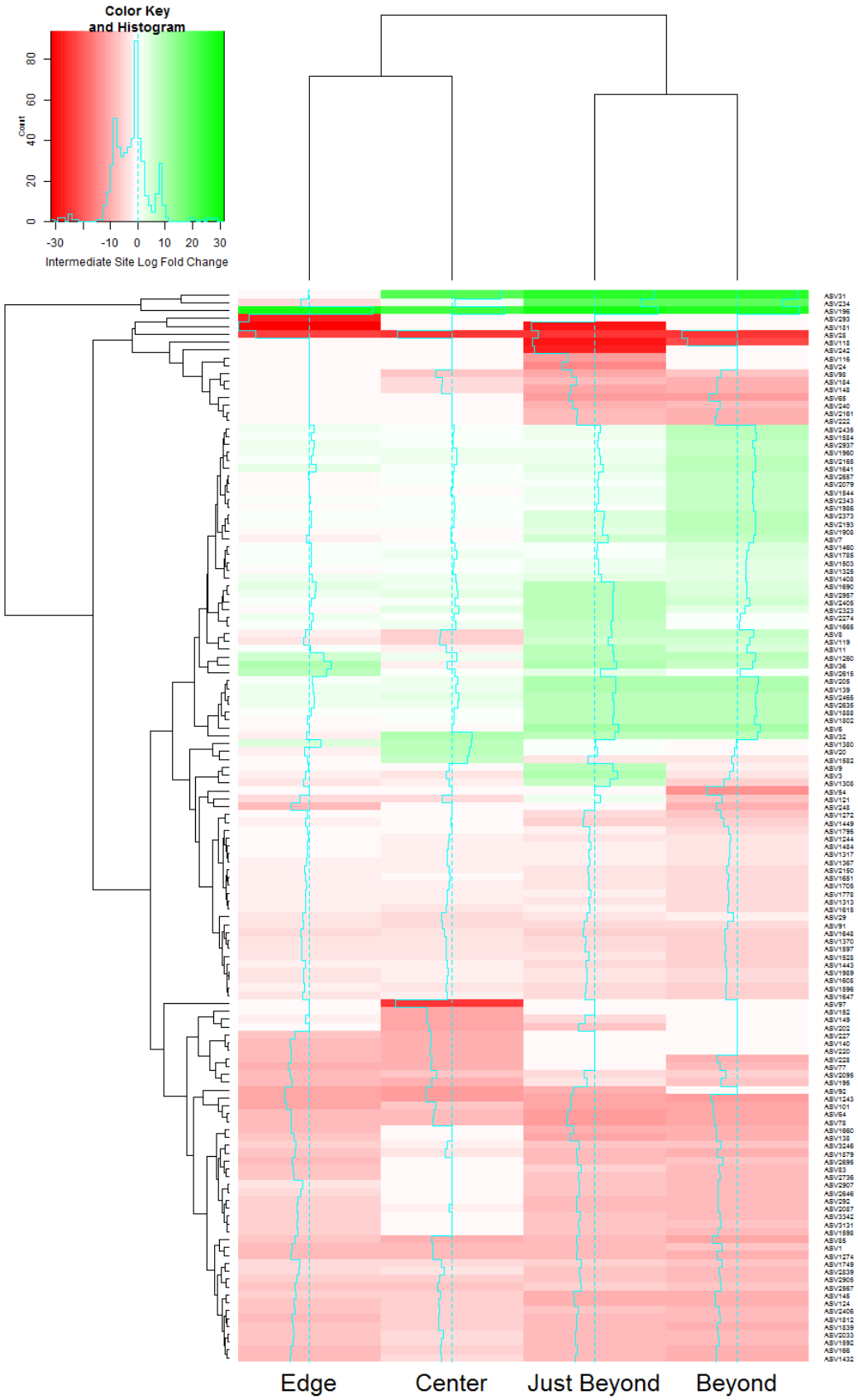
Color Key
and Histogram
Count
0
20
40
60
80
-30
-10
0
10
20
30
Intermediate Site Log Fold Change
Edge
Center
Just Beyond
Beyond

Figure S9. Heatmap including the 136 ASVs which were significantly either over or under abundant in the Intermediate inoculum relative to at least one of the other inoculum source (Center, Edge, Just Beyond, or Beyond). Color indicates the magnitude of the $\log_2$ fold change in abundance of each ASV (names listed on right hand side) when comparing Intermediate inoculum to each of the other four inocula. Red values indicate that the Intermediate inocula was relatively depauperate in that ASV, while green values indicate that the Intermediate inocula was relatively enriched in that ASV. The only ASV for which Intermediate inocula was significantly enriched relative to *all* other inocula was ASV196 (third row from top), indicated by the bright green shading for each pairwise abundance comparison.

Appendix 4

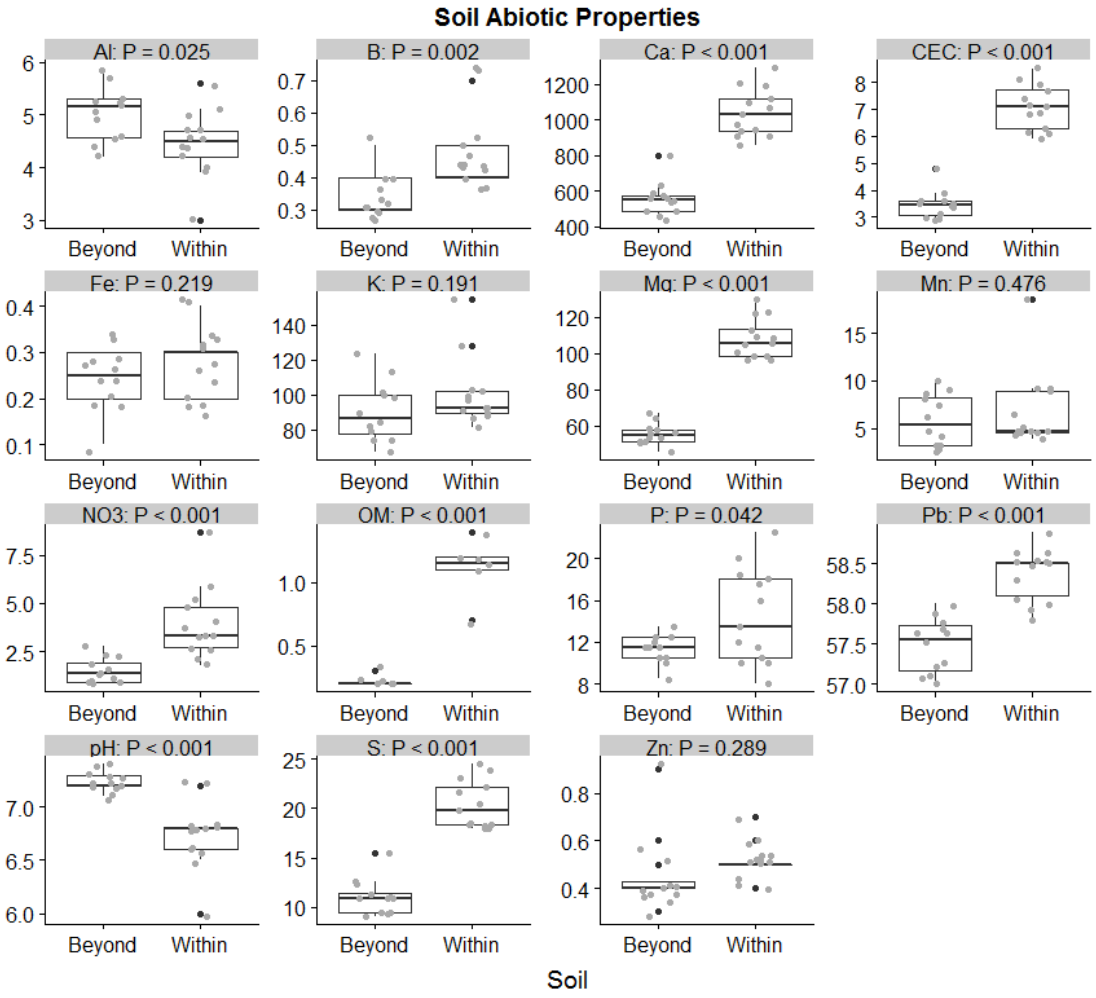

Figure S1. Soil abiotic properties (nutrients, cation exchange capacity, organic matter, and pH) of within and beyond range soils. Nutrient values are in ppm; CEC is in cmole+ / 100g. Panel titles include the unadjusted *P* value for a Student's t-test of differences between soil types.

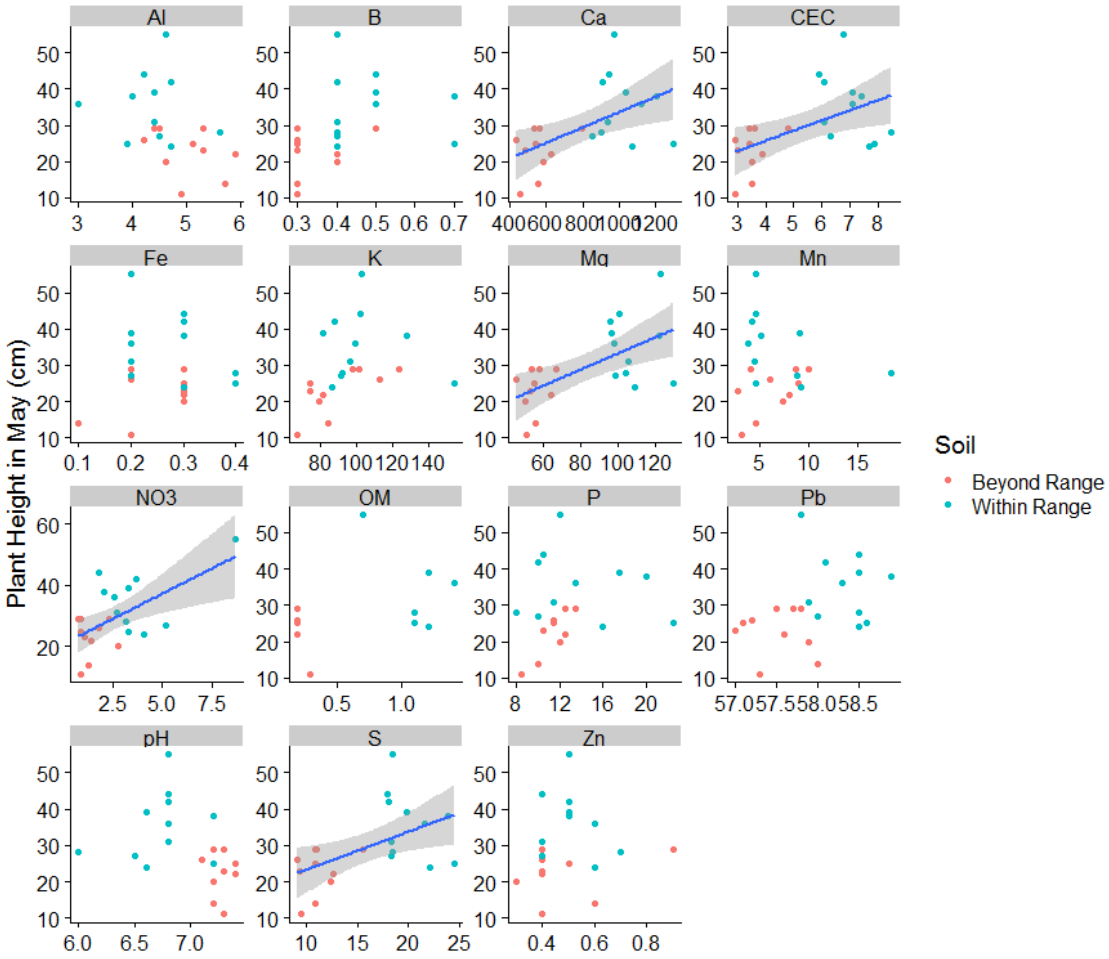

Figure S2. Relationship between soil abiotic properties and plant height in May, using mesocosms exhumed in May in year 2. Panels with blue regression lines (plus 95% confidence bands) have a significant relationship between plant height and nutrient value or CEC. Nutrient values are in ppm; CEC is in cmole+ / 100g.

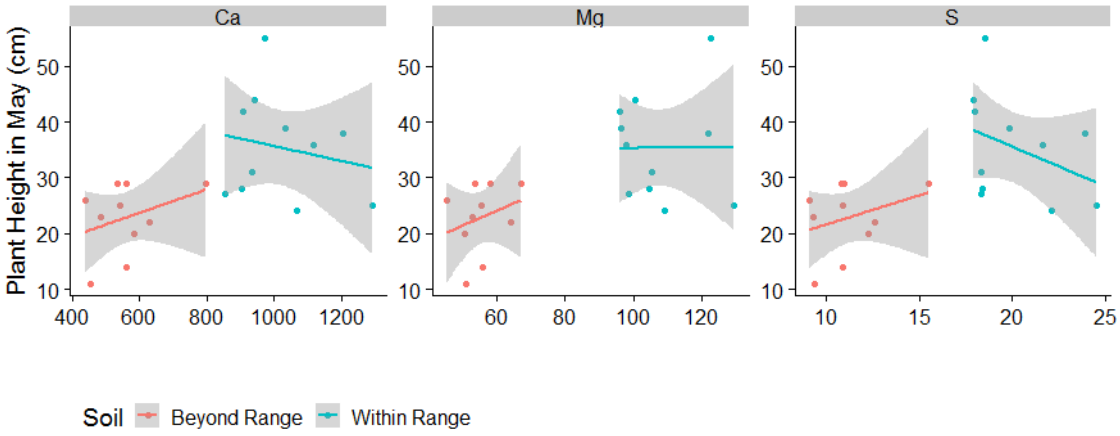

Figure S3. Relationship between Ca, Mg, and S, and plant height in May, using mesocosms exhumed in May in year 2. Regression lines (plus 95% confidence bands) are drawn for each soil type separately. These linear regressions are not significant at α = 0.05 and are shown for illustrative purposes only. Nutrient values are in ppm.